ARTIFICIAL HYBRIDIZATION IN THE GENUS *IMPATIENS*

by

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ABSTRACT

M.Sc. CATHERINE M. MERLIN Plant Science

ARTIFICIAL HYBRIDIZATION IN THE GENUS *IMPATIENS*

The biosystematic relationships of *Impatiens walleriana* Hook. f. and other selected species were examined by means of hybridization experiments and cytological techniques. Both *I. gordonii* Horne and *I. usambarensis* Grey-Wilson hybridized with *I. walleriana*. Meiotic abnormalities of the hybrids were determined by examining pollen mother cells. Meiosis was regular in the majority of cells in both of the hybrids, but *I. walleriana* x *I. gordonii* had more abnormalities than *I. walleriana* x *I. usambarensis*. Because of the meiotic abnormalities in the hybrids and the morphological differences between *I. walleriana* and *I. gordonii*, *I. gordonii* was confirmed as a distinct species. *Impatiens usambarensis* was considered a dubious species because it was morphologically similar to *I. walleriana*, and the hybrid between the two species had fewer meiotic abnormalities. Both *I. gordonii* and *I. usambarensis* could be useful in a horticultural breeding program, because they hybridize easily with *I. walleriana*.

RESUME

M.Sc. CATHERINE M. MERLIN Plant Science

HYBRIDATION ARTIFICIELLE DU GENRE IMPATIENS

Les relations biosystématiques d'Impatiens walleriana Hook f. et des autres espèces choisies sont étudiées par des expériences d'hybridation et des techniques cytologiques. Impatiens gordonii Horne et I. usambarensis Grey-Wilson ont formé des hybrides avec I. walleriana. On a examiné les cellules sporogènes afin de déterminer les anormalités méiotiques des hybrides. La méiose se révela normale dans la majorité des cellules chez les deux hybrides mais le croisement I. walleriana x I. gordonii a produit plus d'anormalités que celui de I. walleriana x I. usambarensis. Or, étant donné les anormalités méiotiques des hybrides et les différences morphologiques obtenues entre I. walleriana et I. gordonii, I. gordonii est confirmé dans son statut d'espèce distincte. Le statue d'espèce de I. usambarensis est remis en question vu la similarité morphologique de la dite espèce avec I. walleriana et que les hybrides entre ces deux espèces conduit à moins d'anormalités méiotiques. Ainsi les deux espèces fourniraient de nouveaux cultivars aux éleveurs parce qu'elles forment aisément des hybrides avec I. walleriana.

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CLAIM TO ORIGINAL RESEARCH

This thesis constitutes the original research of the author. An artificial hybridization program was carried out which included the following species: *Impatiens cinnabarina*, *I. flaccida*, *I. gordonii*, *I. harlandi*, *I. platypetala*, *I. pseudoviola*, *I. sodenii*, *I. usambarensis* and *I. walleriana*. This is the first time that *I. cinnabarina*, *I. harlandi* and *I. usambarensis* have been included in such a study. The hybrid *I. walleriana* x *I. usambarensis* was produced artificially for the first time. Meiotic studies were presented on the hybrids and reciprocals of *I. walleriana* x *I. gordonii* and *I. walleriana* x *I. usambarensis* for the first time.

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Chapter 1

INTRODUCTION

Over the past decade, Impatiens walleriana has become an extremely popular bedding plant (Chong 1979) because of its ability to bloom profusely in a shady location. Zinov'eva-Stahevitch (1981) and Grey-Wilson (1980a) have recently reviewed the biosystematics of the genus Impatiens, but further investigations could provide very useful information for the development of new Impatiens cultivars. Therefore, the purpose of this study was to examine the biosystematic relationships of I. walleriana and other selected species by means of hybridization experiments and cytological techniques.

The I. walleriana aggregate from East Africa, comprising I. walleriana Hk. f., I. usambarensis Grey-Wilson, I. pseudoviola Gilg., I. cinnabarina Grey-Wilson, and I. sodenii Engl. & Warb ex Engl., were investigated. I. gordonii Horne from the Seychelles, I. flaccida Arn. from India and Sri Lanka, I. platypetala var. aurantiaca (Teysn ex Kds) Steen from the Celebes, and I. harlandi Dransfield ined. from Borneo were also included in the study because of their morphological similarity to I. walleriana.

Hybridization experiments were attempted among all the above species in order to determine which species were most closely related.

The hybrids obtained were examined in meiosis to determine chromosome pairing relationships. Other meiotic abnormalities, and pollen viability were also recorded to aid in determining species relationships. The potential of the hybrids and their parents as commercial cultivars was also considered.

Chapter 2

LITERATURE REVIEW

Many Impatiens species are endemics (Grey-Wilson 1980a) and an understanding of Impatiens biogeography aids in the understanding of their biosystematics. A review of the systematics and cytology of the species used in this study also provides valuable background information. A summary of the literature on these topics will be presented.

Grey-Wilson (1980a) feels that the progenitor of the Balsaminaceae and Tropaeolaceae may have existed before the breakup of Gondwanaland. This occurred about a hundred million years ago, at which time India also broke off (Raven and Axelrod 1974). After the breakup of Gondwanaland, the Tropaeolaceae developed in South America but continued to share many points of similarity with the Balsaminaceae. Less than 65 million years ago, the Balsaminaceae arose in East Africa, and migration between Africa, India and Madagascar would have been possible for some time after this (Grey-Wilson 1980a). It is considered unlikely that any part of southeast Asia or Indonesia was part of Gondwanaland (Raven and Axelrod 1974; Grey-Wilson 1980a). The genus Impatiens probably migrated into southeast Asia when India collided with Asia and formed the Himalayas (Grey-Wilson 1980a).

Species of the genus Impatiens seem to fall into two groups. One group occurs in Africa, Madagascar, southern India and Indonesia, and is quite different from the group occurring in the Himalayas and southeast Asia. It is possible that the arid gap between southern India and the Himalayas may have precluded contact between the two groups (Grey-Wilson 1980a). The species in this study belong to the African-Indonesian group.

Around 27 million years ago in the Oligocene, Africa was subjected to cyclical spreading droughts which eliminated numerous taxa and isolated others (Grey-Wilson 1980a; Raven and Axelrod 1974). Today, many species of the African rainforest are common and widespread, unlike the rare and local plant species found in the rainforests of America and southeast Asia (Raven and Axelrod 1974). The opposite seems to be true in the genus Impatiens. Grey-Wilson (1980a) described 109 species native to Africa and pointed out that most of these species were restricted to a small area only, and were frequently endemic to a single mountain or mountain range.

Most Impatiens species are restricted to forest or moist semi-shaded habitats. Many of the endemic species occur in east Africa where the dry season lasts from May to October, and Impatiens are restricted to montane forests. Such a habitat would have a tendency to be isolated on mountain «islands,» and one would expect to find endemics. In west Africa, the dry season is only three months, and consequently, there are more continuous lowland forests. It is not surprising that in west Africa the Impatiens species tend to have a wider range and are fewer

(Grey-Wilson 1980a). Therefore, the high number of Impatiens species can be explained by a combination of geography and ecology.

In her review of the genus Impatiens, Zinov'eva-Stahevitch (1981) found that the major phylogenetic lines were not restricted to phytogeographic regions. She placed all the species in the present study in group 1. The diagnostic characteristics of group 1 are: «type A flower (relatively actinomorphic lepidoptera pollinated) with petals of the alae large, vexillum non-cucullate; shallow, navicular antivexillar sepal with long filliform spur» (Zinov'eva-Stahevitch 1981).

The literature for each species in this study will be reviewed individually. Descriptions of the African species can be found in Grey-Wilson's monograph (Grey-Wilson 1980a). Only pertinent details will be given here.

Impatiens walleriana Hook.

Synonyms: I. sultani Hook.

I. holstii Engl.

(Grey-Wilson 1980a)

The range of I. walleriana was originally thought to be in two areas in east Africa (Grey-Wilson 1980a). The northern area included southeast Kenya, northeast Tanzania and the islands of Zanzibar and Pemba. The southern area consisted of south Malawi, west Mozambique and east Zambabwe (Grey-Wilson 1980a). Grey-Wilson (1980e) later collected plants from Ifakara and Mahenge, and so the range for this species in east Africa now extends in a continuous arc.

Several authors have reported a chromosome number of 2n=16 for I. walleriana (Jones and Smith 1966; Smith 1934; Warburg 1938; Zinov'eva-Stahevitch 1981; Zinov'eva-Stahevitch and Grant 1984, 1985a). With the exception of Smith (1934), none of the above authors found satellite chromosomes in the material they examined. Smith (1934) found one pair of chromosomes with satellites in a plant he called I. sultani but there was no herbarium voucher mentioned for the count. A pair of chromosomes with satellites has also been observed in an I. walleriana plant collected by Dr. Whitehead. (This plant has been given the accession number I-0108 in our collection.)

Warburg (1938) reported 8 bivalents at metaphase I in I. sultani. LeBan and Myers (1980) studied microsporogenesis in male sterile lines of I. sultani. Meiosis was usually normal but lagging chromosomes, chromosome bridges and polyspory were occasionally observed. In the fully male sterile line, microspore abortion occurred previous to pollen mitosis.

Van Went (1981) studied differences between male fertile and male sterile anthers. Meiosis was normal until microspore release from the quartets. In the male fertile lines, the callose divides quickly and completely and the tapetum develops, in contrast to the male sterile lines in which there is some callose division but the tapetum fails to develop. Therefore, the author concluded that the male-sterility was a result of the malfunctioning of an abnormal tapetum (Van Went 1981).

Tara and Namboodiri (1974) found that early meiosis was abnormal in sterile lines of I. sultani. As many as 16 univalents were found

at metaphase I, and the authors felt that these were bivalents that had undergone desynapsis. However, the majority of aberrations appeared after anaphase I, and they included chromosome bridges, lagging chromosomes, unequal separation, and extra micronuclei in the quartet cells.

In 1976, Tara and Namboodiri investigated cytoplasmic furrowing at the end of telophase II. Furrowing was initiated at the same time in normal and abnormal cells but the rate of progression was not synchronous. Three types of cleavage aberration were observed: (1) None took place and a single large spore was formed. This happened rarely. (2) Frequently the first cleavage was completed but the second was never initiated. (3) The second division was initiated but never completed. Wall development began at the appointed time whether cleavage was completed or not, and the authors felt that chromosomes per se do not regulate pollen wall synthesis because even pollen with 0 to 1 chromosome had an exine identical to that of pollen with a normal chromosome complement.

Arisumi (1980a) has obtained hybrids between I. walleriana and a plant he called I. thomasetti (now known to be I. gordonii). The hybrids had features of both parents and bloomed longer than I. gordonii. The backcross with I. walleriana was successful but the one with I. gordonii was not. In other crosses which Arisumi (1980a) attempted, he found I. walleriana did not cross with I. pseudoviola, I. flaccida var. alba, I. repens, I. uguensis (=I. sodenii), I. platypetala, I. platypetala var. aurantiaca, or I. hawkeri sensu lato.

Grey-Wilson (1980a, 1980d) describes putative natural hybrids between I. walleriana and I. usambarensis which occurred only in

disturbed habitats. He never attempted to obtain the hybrid by artificial hybridization, however.

Impatiens usambarensis Grey-Wilson

Impatiens usambarensis has only recently been described by Grey-Wilson (1979). Before this, I. usambarensis was considered part of the natural morphological variation of I. walleriana Hook. f. sensu lato, but Grey-Wilson (1980a) has shown that there are reliable characters which may be used to distinguish the two taxa (Table 2.1).

Zinov'eva-Stahevitch (1981) reports a chromosome number of 2n=16 with one pair of chromosomes with satellites for I. usambarensis. (This is accession number I-0203 in our collection.) (Zinov'eva-Stahevitch and Grant 1984, 1985a).

Impatiens usambarensis is endemic to the Usambara Mountains of northeast Tanzania and is a forest species found growing in moist, shady and semi-shady areas. I. walleriana, on the other hand, prefers more open sites. Grey-Wilson feels that I. walleriana is not native to this region and its introduction to the Usambara region may have made hybridization with I. usambarensis possible (Grey-Wilson 1980a).

Grey-Wilson (1980a) reports that the hybrid of I. walleriana and I. usambarensis has intermediate characteristics between the two species. The extra floral nectaries of the hybrid may be scattered along the petiole or more or less clustered near the top. The lamina has between 5 and 10 pairs of lateral veins and is pubescent beneath, but not as much as in I. usambarensis. The lateral united petals are

22-26 mm long, intermediate between I. walleriana and I. usambarensis. The hybrid is generally associated with disturbed habitats (Grey-Wilson 1980a).

TABLE 2.1. Grey-Wilson's characteristics for separating I. walleriana and I. usambarensis

Character	I. usambarensis	I. walleriana
Leaf lamina	hairy beneath	glabrous beneath
Leaf margin	serrate	crenate
No. pairs of leaf lateral veins	(8)9-14	5-8
Leaf petiole glands	crowded at top	scattered
Length of lateral united petals	(20)24-30 mm	18-25 mm

(After Grey-Wilson 1980a)

Impatiens gordonii Horne

Synonym: I. thomasetti Hook. f.

(Grey-Wilson 1980b)

Impatiens gordonii is a rare species endemic to the Seychelles. It occurs on the island of Mahé and there is a record from Silhouette (Grey-Wilson 1980b). It is found on the edges of forests in rich soils (Baker 1877).

A description and drawing of I. gordonii can be found in Grey-Wilson (1980b). Our accession I-0301 is a cutting from the original plant that was used as a model for the drawing. I. gordonii has white flowers with a long spur and is almost certainly moth-pollinated (Grey-Wilson 1980b).

Impatiens gordonii is most closely allied to I. walleriana and I. usambarensis (Grey-Wilson 1980b). As already noted, Arisumi (1980a) has obtained a hybrid with I. walleriana. However, he was unable to obtain hybrids between I. gordonii and I. pseudoviola, I. uguensis (=I. sodenii), I. flaccida var. alba, I. platypetala, I. platypetala var. aurantiaca, I. repens, and I. hawkeri (Arisumi 1980a).

Impatiens gordonii has a chromosome number of 2n=16 with one pair of chromosomes with satellites (Zinov'eva-Stahevitch 1981; Zinov'eva-Stahevitch and Grant 1984, 1985a). Zinov'eva-Stahevitch and Grant (1985b) found no meiotic abnormalities in I. gordonii, and 8 bivalents were formed at metaphase I (Zinov'eva-Stahevitch 1981).

Impatiens sodenii Engl. & Warb. ex Engl.

Synonyms: I. thomsoni Oliv.
I. uguensis Warb.
I. oliveri GH Wright
I. elgonensis Fries
I. magnifica Schulze
(Grey-Wilson 1980b)

Impatiens sodenii is distributed in Kenya and north and east Tanzania. A colour drawing can be found in Grey-Wilson (1977).

Zinov'eva-Stahevitch (1981) found that I. sodenii had a chromosome number of 2n=16 with 1 pair of chromosomes with satellites. At the quartet stage of meiosis, extra micronuclei were observed (Zinov'eva-Stahevitch 1981; Zinov'eva-Stahevitch and Grant 1984, 1985b).

Arisumi (1980a) attempted crosses between I. sodenii and I. pseudoviola, I. gordonii, I. walleriana, I. flaccida var. alba, I. repens, I. platypetala, I. platypetala var. aurantiaca, and I. hawkeri; but was unsuccessful. However, by using ovule-culture techniques he did obtain a hybrid between I. sodenii and I. flaccida var. alba. The hybrid had light lavender flowers that were almost as large as I. sodenii (Arisumi 1980b).

Impatiens pseudoviola Gilg

Synonyms: I. kwaiensis Gilg

I. filicetorum Fries.

I. hemrichii Schultze

(Grey-Wilson 1980a)

Impatiens pseudoviola is distributed in central and southern Kenya and northern Tanzania.

Impatiens pseudoviola has a chromosome number of 2n=16 with one pair of satellite chromosomes (Arisumi 1980a; Jones and Smith 1966; Zinov'eva-Stahevitch 1981; Zinov'eva-Stahevitch and Grant 1984, 1985a). No meiotic abnormalities were observed by Zinov'eva-Stahevitch and Grant (1985b).

Arisumi (1980a) found that I. pseudoviola was self-incompatible and did not cross with I. gordonii, I. sodenii, I. walleriana, I. flaccida var. alba, I. repens, I. platypetala, I. platypetala var. aurantiaca, or I. hawkeri. However, Gilg (1909), from a study of herbarium specimens, considered that I. pseudoviola may have hybridized

with I. kilimanjari Oliv. to give rise to the species I. lateritia Gilg. Grey-Wilson (1980d) supported this opinion from a study of field collections which he made.

Impatiens cinnabarina Grey-Wilson

Impatiens cinnabarina is endemic to the Kimboza area on the eastern slopes of the Uluguru Mountains of Tanzania in an interesting area of metamorphosed limestone (Grey-Wilson 1980a). A description of the species may be found in Grey-Wilson (1979, 1980a).

Zinov'eva-Stahevitch (1981) found that I. cinnabarina had a chromosome number of 2n=16 with one pair of satellite chromosomes (Zinov'eva-Stahevitch and Grant 1984, 1985a). No meiotic abnormalities were observed (Zinov'eva-Stahevitch and Grant 1985b).

There is no record of hybridization studies involving I. cinnabarina, but Grey-Wilson (1980a) reported that he considers I. cinnabarina may be of hybrid origin with I. walleriana and I. ?hamata as the putative parents.

Impatiens flaccida Arn.

Impatiens flaccida originated in southern India and Sri Lanka, but has become naturalized in many other areas, e.g., on Maurice and La Réunion on the Mascareignes (Grey-Wilson 1980c).

Arnon (ex Hooker 1872) describes I. flaccida as a slender, erect, sparingly-branched plant with ovate or lanceolate leaves with a crenate margin. The standard and wings are broad and bilobed, the sepals are

ovate and there is a long slender spur. The capsule is glabrous and the seeds are globose and tubercled (Arnon ex Hooker 1872). Grey-Wilson (1980c) has published a drawing of I. flaccida showing a long slender fruit. However, Dr. A. E. Zinov'eva-Stahevitch (Biosystematics Research Institute, Agriculture Canada, Ottawa, Ontario) has informed me that in the wild, I. flaccida may have a more gibbous fruit (personal communication). Trimen and Hooker (1893) also describe I. flaccida as having a gibbous fruit.

Trimen and Hooker (1893) give the range of I. flaccida to be Sri Lanka, Java and southern India. However, they were not sure that the plant called I. flaccida in India was the true plant. They also mentioned that there was a variety with pure white flowers that had long been under cultivation in England under the name «I. platypetala var. alba.» This could be I. flaccida var. alba.

I. flaccida has a chromosome number of 2n=16 with one pair of satellite chromosomes (Arisumi 1980a; Jones and Smith 1966; Zinov'eva-Stahevitch 1981; Zinov'eva-Stahevitch and Grant 1985a) as does I. flaccida var. alba (Arisumi 1980a; Zinov'eva-Stahevitch 1981; Zinov'eva-Stahevitch and Grant 1985a). Jones and Smith (1966) observed seven bivalents at metaphase I in I. flaccida. Zinov'eva-Stahevitch (1981) observed precocious separation at metaphase I and chromatid bridges at telophase I. Aberrant quartets with micronuclei and extra nucleoli were also observed (Zinov'eva-Stahevitch and Grant 1985b).

Arisumi (1977) reported that he had obtained a hybrid between I. flaccida and an I. platypetala from Borneo. Unfortunately, the

I. platypetala in question was mislabelled and was actually I. flaccida so this was not an inter-specific cross (Dr. T. Arisumi, USDA, Beltsville, Maryland, personal communication).

I. flaccida var. alba did not cross with I. pseudoviola, I. gordonii, I. sodenii, I. walleriana, I. platypetala var. aurantiaca, I. platypetala, I. repens, or I. hawkeri (Arisumi 1980a). With ovule-culture techniques, he obtained hybrids between I. flaccida var. alba and I. uguensis (=I. sodenii) from Africa and I. repens from Sri Lanka (Arisumi 1980b).

The I. flaccida var. alba x I. sodenii hybrid has already been described under I. sodenii. I. flaccida var. alba x I. repens had flowers with light yellow throats and many pale pink-violet petals in contrast to I. repens which has deep yellow helmet-shaped flowers (Arisumi 1980b).

Impatiens platypetala Lindl.

Backer and Van den Brink (1963) describe I. platypetala as having an erect stem which is much thickened at the nodes. The leaves are opposite or in whorls; ovate-oblong or laceolate in shape with a crenulate to serrulate or coarsely serrate margin. The corolla may be red-purple, dark red, dark pink, pale pink, and rarely white or salmon orange. The authors describe four subspecies after Van Steenis. I. platypetala var. nematoceras is a frail, much-branched plant with small flowers up to 2 cm in diameter, and pink to purple in colour. I. platypetala var. platypetala has large purple flowers 2.5-6 cm in

diameter. I. platypetala var. nivea has plain white flowers, sometimes without the spur. Dr. A. E. Zinov'eva-Stahevitch (Biosystematics Research Institute, Agriculture Canada, Ottawa, Ontario) has informed me that this plant has some pigmentation, and cannot be confused with I. flaccida var. alba (personal communication). The last variety, I. platypetala var. aurantiaca is from the Celebes and has salmon orange flowers.

Van Steenis (1949) felt that I. platypetala and I. platypetala var. nematoceras were regional subspecies because they excluded each other geographically and differed in habitat and size. I. platypetala var. aurantiaca from the Celebes differed only in the colour of the flower, and not by structural changes, and has the same ecology as I. platypetala from Java. (The line drawing of I. platypetala var. aurantiaca looks very similar to the commercial cultivar 'Tangerine' that was selected from I. platypetala var. aurantiaca.)

Clevenger (1971) feels that I. platypetala var. aurantiaca should be given specific rank because it has a unique pigment, namely aurantidin, that has not been discovered anywhere else in the plant kingdom, and its precursors are not found in I. platypetala var. platypetala.

Impatiens platypetala var. platypetala has two chromosomal races: 2n=14 (Lee 1967; Zinov'eva-Stahevitch 1981; Zinov'eva-Stahevitch and Grant 1985a), and 2n=16 (Arisumi 1975, 1978, 1980a; Beck et al. 1974; Zinov'eva-Stahevitch 1981; Zinov'eva-Stahevitch and Grant 1985a). Zinov'eva-Stahevitch (1981) also observed a quartet cell with five nuclei.

Impatiens platypetala var. aurantiaca also has two chromosomal races. Khoshoo (1955, 1957) reported n=7 and Zinov'eva-Stahevitch (1981) 2n=14 (Zinov'eva-Stahevitch and Grant 1985a); whereas the commercial cultivar 'Tangerine' selected from I. platypetala var. aurantiaca has a chromosome number of 2n=8 (Arisumi 1975, 1978; Beck et al. 1974).

Arisumi (1974, 1980a) has reported that I. platypetala var. platypetala (2n=16) and I. platypetala var. aurantiaca (2n=8) hybridize, and the hybrid has 2n=12. Both I. platypetala var. platypetala (2n=16) and I. platypetala var. aurantiaca (2n=8) hybridize with I. hawkeri (2n=32) (Arisumi 1974, 1980a). I. platypetala var. platypetala and I. hawkeri sensu lato have chromosomes morphologically similar in size and shape, but the chromosomes of I. platypetala var. aurantiaca are quite different in morphology (Arisumi 1973, 1978).

Thakur (1980) found that I. platypetala var. platypetala could be distinguished from I. linearifolia (syn. for I. hawkeri sensu lato) by the anthocyanins present.

Impatiens harlandi Dransfield ined.

Very little has been published on I. harlandi. The species originates in Borneo and Zinov'eva-Stahevitch and Grant (1984, 1985a) reported a diploid chromosome number of 2n=12.

Pollen studies

Hunynh (1968) tried to arrange the species within the genus Impatiens based on pollen morphology. He considered that there were two lines of evolution in Impatiens. One line consisted of the species with 4-colpate rectangular pollen; and the other, species with 4-colpate (sometimes 3 or 5) square pollen. He also mentioned that there is often an increase or decrease in the number of apertures, particularly in the 4-colpate rectangular pollen group. Supplementary apertures could be recognized by a different paracolpial reticulum. In some species, 4-colpate square and 3-colpate triangular pollen could be found in the same flower (Hunynh 1968). Grey-Wilson (1980d) also noted this fact.

Hunynh (1968) considered that there was no affinity between I. walleriana and I. pseudoviola because I. walleriana had 4-colpate rectangular pollen and I. pseudoviola had 3-colpate triangular pollen. I. magnifica and I. oliveri (both synonyms for I. sodenii) also had 3-colpate triangular pollen.

There were pollen affinities between I. platypetala from Celebes (I. platypetala var. aurantiaca) and the New Guinea species, I. hawkeri, I. herzogii, and I. mooreana (I. hawkeri sensu lato). All had 4-colpate square pollen of a type denoted «Archipel Malais» (Hunynh 1968). Khoshoo (1957) found that I. platypetala var. aurantiaca had round pollen instead of square.

Grey-Wilson (1980d) examined pollen in hybrids between the two pollen groups of Hunynh (1968). I. pseudoviola with 3-colpate

triangular hybridized with I. kilimanjari which had 4-colpate rectangular pollen. I. austrotanzanica with 4-colpate rectangular pollen crossed with I. rubromaculata var. grandiflora which had 3-colpate triangular or 4-colpate square pollen. The pollen of the hybrids exhibited the shape of one of the parents and often inherited surface details from the second parent (Grey-Wilson 1980d).

Conclusion

Grey-Wilson and Zinov'eva-Stahevitch have recently reviewed the systematics of the Impatiens species used in this study, and their work has provided valuable background information. With the exception of Grey-Wilson's (1980a) mention of a putative natural hybrid between I. walleriana and I. usambarensis, Arisumi is the only researcher who has produced hybrids between any of the species used in this study. However, Arisumi did not examine the meiotic configurations in any of his hybrids.

Chapter 3

MATERIALS AND METHODS

The species used in this study were: the I. walleriana aggregate from east Africa, including I. walleriana Hk. f., I. usambarensis Grey-Wilson, I. pseudoviola Gilg, I. cinnabarina Grey-Wilson, and I. sodenii Engl. & Warb. ex Engl.; I. gordonii Horne from the Seychelles; I. flaccida Arn., and I. flaccida var. alba from India and Sri Lanka; I. platypetala var. aurantiaca (Teysm ex Keds) Steen from Celebes; and I. harlandii Dransfield ined. from Borneo. A list of accessions used in this study is given in Table 3.1

The Impatiens plants were grown in a shady greenhouse, without supplementary light, that was maintained at 20° ± 5°C in the winter months. In the summer, the temperature was lowered by using whitewash on the windows, high-speed fans, and soaking the floor with water a few times a day.

Plants were grown in clay pots containing 1 part peat, 1 part pasteurized soil, and 1 part sand or perlite. A portion of dolomitic limestone was also added to the potting mixture. Plants were fertilized once a week with half-strength 20-20-20 fertilizer. During the winter months, when growth slowed, it was only necessary to fertilize once every two weeks.

TABLE 3.1. *Impatiens* accessions used in this study

Accession number	Species	Origin	Source	Collector
I-0101	*I. walleriana*	Africa	Kew(026-76)00189*	GW**
I-0102	*I. walleriana*	Africa	Kew(129-76)00894	GW
I-0103	*I. walleriana*	Africa	Kew(105-79)01056	GW & Cribb
I-0104	*I. walleriana*	Africa	Kew(026-76)00189*	GW
I-0105	*I. walleriana*	Africa	Kew(129-76)00894	GW
I-0106	*I. walleriana*	Africa	Kew(129-7)00894	GW
I-0107	*I. walleriana*	Africa	Kew	
I-0111	*I. walleriana*	Africa	commercial cultivar	
I-0108	*I. walleriana*	Africa	Kew(240-73)02294	Whitehead
I-0201	*I. usambarensis*	Africa	Kew(105-79)01071	Cribb & GW
I-0202	*I. usambarensis*	Africa	Kew(105-79)01070	Cribb & GW
I-0203	*I. usambarensis*	Africa	Kew(026-76)00182	Cribb & GW

(table continued)

TABLE 3.1 (continued)

Accession number	Species	Origin	Source	Collector
I-0301	*I. gordonii*	Seychelles	Kew(260-74)02258	Roche
I-0401	*I. cinnabarina*	Africa	Kew(129-76)00904	GW
I-0501	*I. pseudoviola*	Africa	Kew(026-76)00179	Cribb & GW
I-0502	*I. pseudoviola*	Africa	Kew(345-81)08139	Cribb
I-0601	*I. harlandi*	Borneo	Kew(383-79)03631	Dransfield
I-0701	*I. sodenii*	Africa	Kew(100-76)00748	Cribb & GW
I-0702	*I. sodenii*	Africa	Kew(709-63)070901	Wandby
I-0801	*I. flaccida* var. *alba*	India & Sri Lanka	Arisumi via C. Nozzolillo	
I-0802	*I. flaccida*	India & Sri Lanka	Arisumi via C. Nozzolillo	
I-0902	*I. platypetala*	Java	Arisumi via C Nozzolillo	P.I.349629
I-1001	*I. platypetala* var. *aurantiaca*	Celebes	A.E.Zinov'eva-Stahevitch	See source

*When different Accession numbers have matching Kew reference numbers, they are different plants from the same population.

**GW = Grey-Wilson.

Every 4-8 months plants were repotted or cuttings taken. The cuttings were rooted in the greenhouse in a styrofoam flat containing a mixture of perlite and vermiculite. No growth hormone was used to promote rooting because it was not necessary.

Some of the plants developed viral symptoms. Dr. J. F. Peterson (Virologist, Macdonald College) isolated Cucumber Mosaic Virus from one of the affected plants (personal communication). This virus had been previously reported in I. sultani (=I. walleriana) by Herold (1964). As soon as it became apparent that a virus might be present, all cutting and trimming was done with a sterile scalpel that was flamed in 95% ethanol and dipped in milk. Unfortunately, by this time the virus had already infected a large number of plants and became a significant problem towards the end of the study because the affected plants failed to grow and some died. Figures 3.1 and 3.2 show some plants exhibiting viral symptoms.

Because Impatiens are very susceptible to pesticides, insecticide spraying was only done when absolutely necessary, and malathion was never used because it has been known to cause complete defoliation of Impatiens (Forsyth et al. 1969). Biological pest management was attempted at various times, even though it was very time-consuming. Aphids were controlled by Aphidoletes aphidimyza Rond., obtained from Linda Gilkeson of the Department of Entomology, Macdonald College; or by a parasitic wasp, Aphidius matricariae Haliday, that occasionally entered the greenhouse. Spider-mites were controlled by a predator mite, Phytoseiulus persimilis, the best source of which was found to be

Figure 3.1. I. walleriana (I-0103) showing viral symptoms

Virus-infected on left, healthy on right

Figure 3.2. I. leschenaultii (I-1101) showing viral symptoms

Applied Bionomics Ltd., Sidney, British Columbia. Mealy-bugs were controlled by trimming the infected portion of the plant, or by throwing out badly infested plants. Cuttings were rooted in the greenhouse instead of the mist-frame so they would not become contaminated by mealy-bugs. If the insect population in Impatiens greenhouse constituted a threat to other sections of the greenhouse, the plants were sprayed with Pentac, Ambush or Pirimor, and at such times biological control had to be abandoned.

Procedure for making crosses

1. A flower, post-anthesis, in which the anthers were starting to separate, was chosen.
2. Pollen was removed from the flower with a paintbrush to prevent selfing. A separate paintbrush was used for each accession.
3. The flower was bagged with a drawstring crossing-bag (8 x 6 cm) made from Indian cotton or a Kimwipe.
4. The androecium usually fell off in 1-2 days to reveal a gynoecium unexposed to pollen.
5. The stigma was considered fertile when it had assumed an open star shape (see Grey-Wilson 1980a, p.9). The cross was made and labelled and the crossing-bag was replaced.
6. Four or five days after pollination the crossing-bag was permanently removed.
7. Successful crosses were bagged with a plastic sandwich bag (21 x 17 cm) and loosely tied with a twist-tie before the pod exploded.
8. Putative hybrid seeds were placed in an envelope and stored in a cool dry place.

Procedure for the germination of seeds

1. Seeds were surface-cleaned by wrapping them in a Kimwipe and placing them in cool running water for three hours.
2. Washed seeds were placed on 9 cm glass petri dishes with two pieces of filter paper placed in the bottom of the dish and moistened with 3 ml of 2% KNO_3, following the procedure described in Simmonds (1980).
3. The petri dishes were placed in a growth chamber with a photoperiod of 16 hours and a day/night temperature of 25/20°C.
4. Plates were checked weekly and germinated seeds were transferred to cardboard flats containing 1 part sieved peat, 1 part vermiculite, and 1 part perlite. Flats were placed in the greenhouse if the temperature was below 30°C.
5. Seedlings that survived were transferred to individual clay pots and labelled, using the accession numbers of the parents, with the accession number of the female parent first, according to convention. Sibling plants from the same cross were differentiated by the addition of A, B, C, or D to the accession number combinations.

Many seedlings were lost as a result of damping-off, using the above procedure. This problem was alleviated by eliminating the petri-dish stage, and placing the washed seeds directly into flats. In the summer, the flats were placed in an air-conditioned laboratory until the seeds had germinated, because it had been noted that high temperatures can induce secondary dormancy in Impatiens (Personal communication, Dr. J. Simmonds, Agriculture Canada, Ottawa, Ontario). At other times the flats were placed immediately in the greenhouse.

Procedure for staining root-tips

1. Cuttings were placed in a propagating box for about two weeks to obtain good root-tip growth. A greenhouse temperature of 22-30°C provided roots with actively dividing cells.
2. Healthy root-tips were placed in 0.003 M 8-hydroxyquinoline for three hours in the dark. (I. flaccida and I. platypetala were pretreated for only two hours as it was found that three hours greatly contracted the chromosomes.)
3. The root-tips were rinsed in three changes of tap water and fixed in 4 ml of Carnoy's 3:1 (95% alcohol:glacial acetic acid) fixative overnight.
4. Root-tips were rinsed and transferred to 70% ethanol until needed.
5. Root-tips were rinsed and hydrolyzed in 1 N HCl in a 60°C water-bath for 12 minutes to obtain completely stained chromosomes.
6. Root-tips were rinsed and stained by the Feulgen procedure for 2-4 hours in the dark. Root-tips were considered stained by visual observation when they turned pink. It was considered that staining by the Feulgen technique gave better preparations than the aceto-orcein methods described in Ghosh and Bhanji (1982) which were tried.
7. Root-tips were rinsed and placed in a 2% pectinase solution for 1-1.5 hours to aid with maceration.
8. Root-tips were rinsed and transferred to 45% acetic acid for 15 minutes.
9. Root-tips were rinsed and stored in water and examined within 48 hours. Storing stained root-tips in 70% ethanol stained the cytoplasm and this interfered with chromosome observation.

Preparation of root-tip slides

1. A well-stained tip was placed in a drop of 45% acetic acid on a slide, and the meristematic region was teased apart with two clean dissecting needles.
2. The preparation was covered with an 18 mm x 18 mm no.1 coverslip.
3. The slide was covered with a Kimwipe, and two corners of the coverslip were secured with two fingers while the slide was gently tapped and firmly pressed with a cork.
4. If necessary, the cytoplasm was cleared by gently heating the slide in a flame from an alcohol burner.
5. The slide was examined under phase contrast or using Nomarsky interference contrast optics. If the preparation was satisfactory, the slide was made semi-permanent by sealing it with Fisher 'Tissuemat.'
6. Photomicrographs were taken with a Zeiss Photomicroscope with oil on the condenser, using either a 40x-oil phase objective or a 100x-oil objective using phase contrast or Nomarski interference contrast optics.

Procedure for staining flower buds

1. Flower buds with spurs up to 4 mm in length were harvested on a sunny day between 09:00 and 12:00 hours. (This yielded the greatest number of buds at metaphase I.)
2. Buds were fixed in 10 ml of Carnoy's 3:1 fixative. (Carnoy's 6:3:1 with chloroform was tried on a number of occasions but it usually gave a preparation with bubbles in the cytoplasm that obscured the chromosomes.)

3. Buds were transferred to 70% ethanol until needed.
4. Buds were rinsed and hydrolyzed in 1N HCl in a 60°C water bath for 20-25 minutes.
5. Buds were rinsed and then stained by the Feulgen procedure in the refrigerator for 2-6 days. (Snow's alcoholic carmine, acetocarmine and acetoorcein were all tried, but the most satisfactory results were obtained using the Feulgen technique.)
6. Following staining, buds were stored in water and examined within 24 hours. Storing stained buds in 70% ethanol caused the cytoplasm to become darkly stained, thus obscuring the chromosomes.

Preparation of meiotic slides

1. The pollen mother cells were gently released from the anthers into a drop of 45% acetic acid. The rest of the anther was discarded.
2. The preparation was covered with an 18 mm x 18 mm no.1 coverslip.
3. The cells were immediately examined to determine the stage. Cells in the meiotic stages described below were prepared for observation as follows:

   A slide with cells in the quartet stage was sealed with Fisher's 'Tissuemat' without heating or tapping because this shattered the cell membrane. Quartet cells were examined under light or Nomarsky optics and the best photomicrographs were taken using Nomarsky interference contrast optics.

   A slide where the cells were in late pachytene to teleophase II was firmly tapped and the coverslip pressed with a cork. The cytoplasm was cleared by heating the slide in a flame from an

alcohol burner, and the coverslip was sealed with Fisher's 'Tissuemat.' Photomicrographs were taken with a Zeiss photomicroscope using a 40x-oil objective and light optics, or using a 100x-oil objective and Nomarsky interference contrast optics.

Pollen

Pollen from freshly opened flowers was stained overnight with fast green in lactophenol. The number of stained and unstained pollen grains was determined using a magnification of 125x. Pollen shape and number of apertures were determined using a magnification of 500x.

Chapter 4

RESULTS OF THE HYBRIDIZATION EXPERIMENTS

A diagrammatic representation of the hybridization experiments performed is given in Figure 4.1. The numbers of successful and unsuccessful crosses may be found in Table 4.1. From a total of 50 attempted crosses among nine species, only two interspecific hybrids were obtained: I. walleriana x I. usambarensis (and the reciprocal cross) and I. walleriana x I. gordonii (and the reciprocal cross). Detailed results of the crosses with individual species follow.

Hybridization was successful between I. walleriana and I. gordonii, and I. walleriana and I. usambarensis, as previously mentioned. Eight crosses were attempted between I. walleriana and I. gordonii, and two hybrids were produced (I-0103 x I-0301 and I-0106 x I-0301). From 21 attempts to cross I. walleriana and I. usambarensis, four hybrids were obtained (I-0101 x I-0201, I-0103 x I-0202, I-0103 x I-0203, and I-0106 x I-0201). I. walleriana did not cross with I. pseudoviola, I. flaccida, I. sodenii, I. platypetala var. aurantiaca or I. harlandi.

In the case of I. gordonii and I. walleriana, four out of five crosses produced hybrids between these two species (I-0301 x I-0102, I-0301 x I-0104, I-0301 x I-0105, and I-0301 x I-0111). I. gordonii did not cross with I. usambarensis or I. sodenii.

Figure 4.1. Summary of the hybridization experiments performed.

Solid lines indicate successful crosses;
broken lines indicate unsuccessful crosses;
arrow points towards female parent.

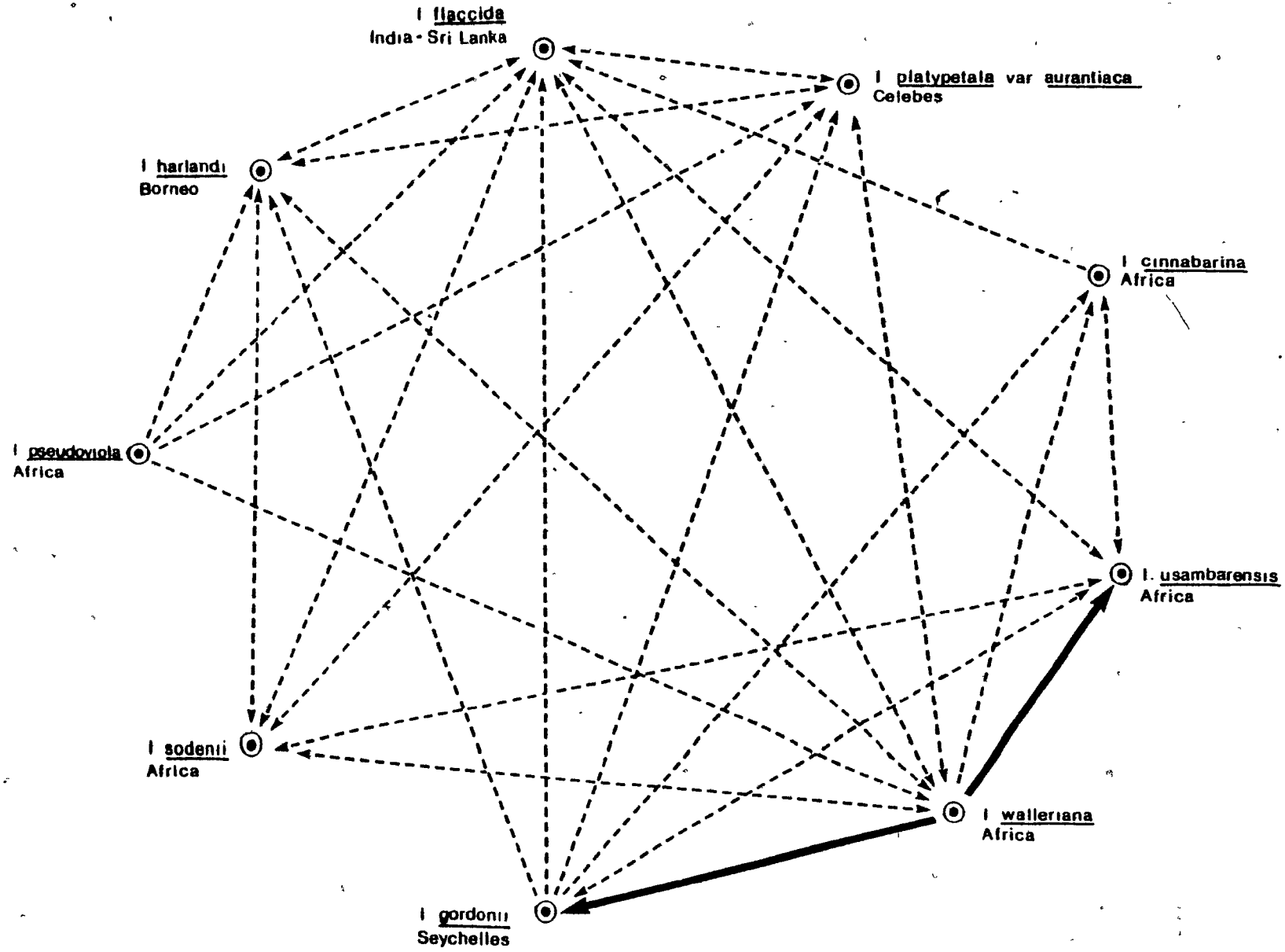

I flaccida
India - Sri Lanka
I platypetala var aurantiaca
Celebes
I harlandi
Borneo
I cinnabarina
Africa
I pseudoviola
Africa
I. usambarensis
Africa
I sodenii
Africa
I walleriana
Africa
I gordonii
Seychelles

TABLE 4.1. Proportion of successful/unsuccessful crosses

	I. walleriana	*I. usambarensis*	*I. gordonii*	*I. cinnabarina*	*I. sodenii*	*I. pseudoviola*	*I. flaccida*	*I. platypetala* var. *aurantiaca*	*I. harlandi*
I. walleriana	27/61	4/21	2/8	-	0/15	0/14	0/14	0/8	0/6
I. usambarensis	8/22	5/9	0/2	0/2	0/6	0/4	0/3	0/2	0/2
I. gordonii	4/5	0/1	0/1	-	0/2	-	-	-	-
I. cinnabarina	0/2	0/2	0/1	-	-	-	-	-	-
I. sodenii	0/13	0/1	0/2	-	2/3	0/2	0/2	0/1	0/1
I. pseudoviola	-	-	-	-	-	0/1	-	-	-
I. flaccida	0/10	0/4	0/2	0/1	0/2	0/3	4/4	0/1	0/1
I. platypetala var. *aurantiaca*	0/7	0/2	0/1	-	0/2	0/2	0/2	1/1	0/1
I. harlandi	0/6	0/3	0/1	-	0/1	0/1	0/2	0/1	0/1

Eight out of 22 crosses produced hybrids between I. usambarensis and I. walleriana (I-0203 x I-0103, I-0201 x I-0103, I-0201 x I-0104, I-0201 x I-0111, I-0202 x I-0102, I-0203 x I-0106, and I-0203 x I-0102). I. usambarensis did not cross with I. pseudoviola, I. cinnabarina, I. flaccida, I. sodenii, I. platypetala var. aurantiaca, or I. gordonii.

To summarize, 6 out of 12 hybridization attempts resulted in hybrids between I. walleriana and I. gordonii; and only 12 out of 43 attempted crosses resulted in hybrids between I. walleriana and I. usambarensis. However, these proportions are not significantly different at $p = 0.10$ using the chi-square distribution or G-test, so the higher proportion of successful crosses for I. walleriana x I. gordonii is likely due to chance, and not due to a greater affinity between I. walleriana and I. gordonii than between I. walleriana and I. usambarensis.

I. cinnabarina did not cross with I. walleriana, I. gordonii, or I. usambarensis.

I. sodenii did not cross with I. walleriana, I. usambarensis, I. pseudoviola, I. platypetala var. aurantiaca, I. flaccida, I. gordonii or I. harlandi.

I. harlandi did not cross with I. walleriana, I. usambarensis, I. pseudoviola, I. platypetala var. aurantiaca, I. gordonii, I. sodenii or I. flaccida.

I. flaccida var. alba (I-0801) and I. flaccida (I-0802) crossed readily; thus helping to confirm that I-0802 is I. flaccida, and not I. platypetala as originally labelled. I. flaccida did not cross

with I. harlandi, I. sodenii, I. gordonii, I. walleriana, I. usambarensis, I. pseudoviola, I. cinnabarina, or I. platypetala var. aurantiaca.

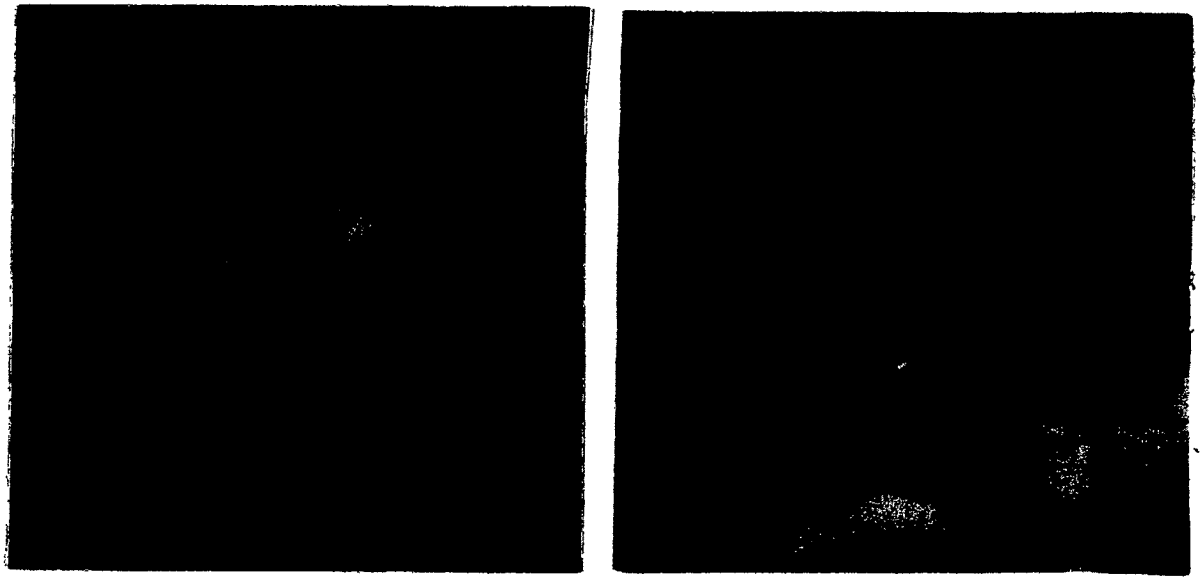
A description of the I. walleriana, I. gordonii, and I. usambarensis accessions can be found in Table 4.2, and colour photographs can be found in Figures 4.2, 4.3 and 4.4. The inter-specific hybrids are described in Table 4.3, and colour photographs can be found in Figures 4.5 and 4.6.

TABLE 4.2. Descriptions of I. walleriana, I. usambarensis, and I. gordonii accessions

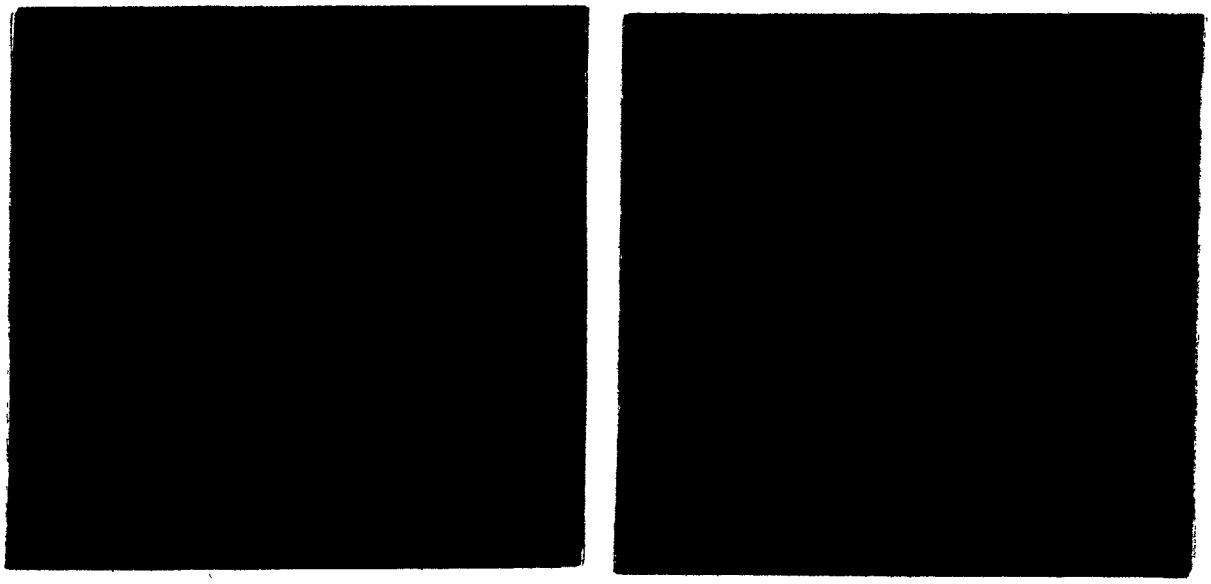
Accession number	Flower colour	RHS* flower colour number	Position extrafloral nectaries	Leaf margin	No. pairs leaf lateral veins	Rank leaf pubescence**	
						top	bottom
I. walleriana							
I-0101	dark red	57A	scattered	crenate	7-8	1	1
I-0102	salmon	50A	scattered	crenate	7	0	0
I-0103	pink	68B	scattered	crenate	6-7	0	0
I-0104	dark red	66A	scattered	crenate	7-9	1	1
I-0105	salmon	40A	scattered	crenate	7-8	0	0
I-0106	pink	67C	scattered	crenate	5-6	0	0
I-0107	pink	68A	scattered clumped	crenate	5-6	0	0
I-0111	purple	74A	scattered	crenate	5-6	2	1
I. usambarensis							
I-0201	deep pink	52A	clumped	serrate & crenate	7-8	2	2
I-0202	coral pink	50B	clumped	serrate	6-7	2	2
I-0203	orange	32A	clumped	serrate & crenate	6-8	3	3
I. gordonii							
I-0301	white		absent	crenate	5-6	0	0

*RHS - Royal Horticultural Society

**0=glabrous; 1=very slightly pubescent when seen under the dissecting microscope; 2=pubescent to the naked eye; 3=heavily pubescent.

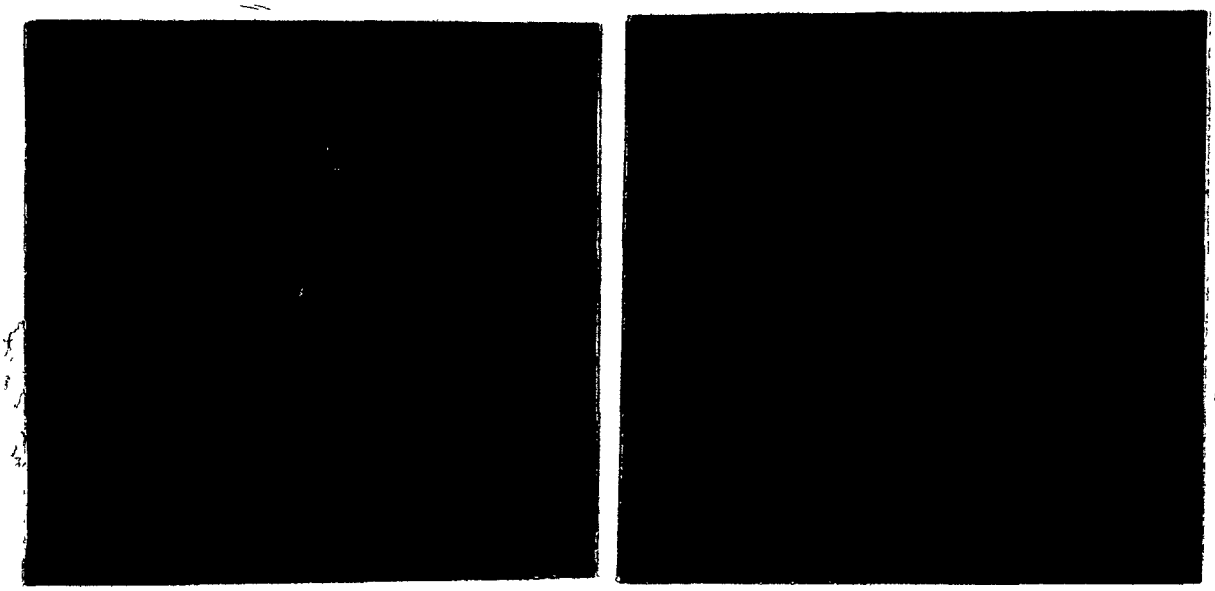


(a) I-0103 (b) I-0104



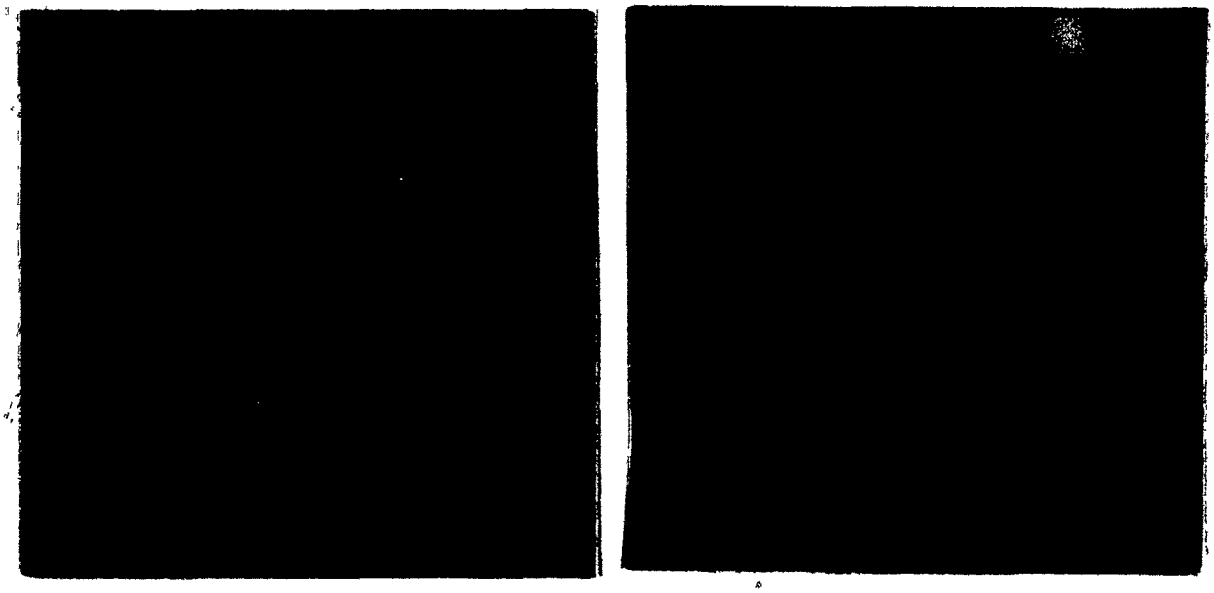
(c) I-0105 (d) I-0111

Figure 4.2. Flowers of I. walleriana accessions



(a) I-0106
I. walleriana

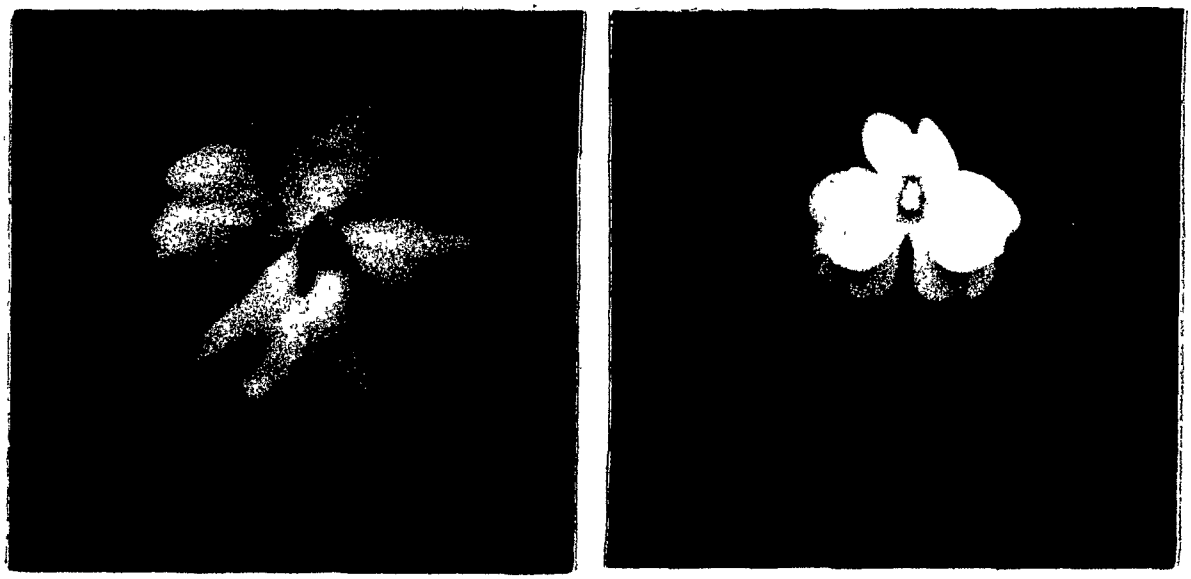
(b) I-0201
I. usambarensis



(c) I-0202
I. usambarensis

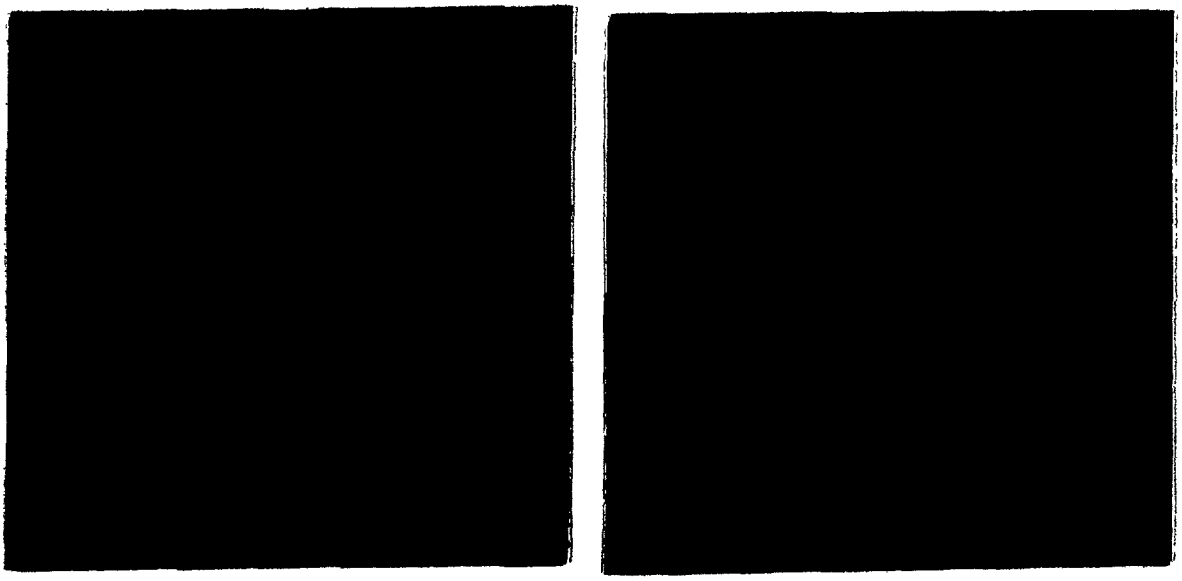
(d) I-0203
I. usambarensis

Figure 4.3. Flowers of Impatiens accessions



(a) I-0301
I. gordonii

(b) I-0801
I. flaccida var. *alba*



(c) I-0802
I. flaccida

(d) I-1001
I. platypetala var. *aurantiaca*

Figure 4.4. Flowers of *Impatiens* accessions

TABLE 4.3 Descriptions of the hybrids obtained

Accession number	Flower colour	RHS* flower colour number	Position of extrafloral nectaries	Leaf margin	No. pairs leaf lateral veins	Rank leaf pubescence**	
						top	bottom
I. walleriana x *I. usambarensis*							
I-0101 x I-0201	deep pink	52A	scattered clumped	serrate	8-9	2	2
I-0103 x I-0202	salmon	44D	scattered	crenate	9-10	0	0
I-0106 x I-0201	deep pink	58B	-	-	-	-	-
I. usambarensis x *I. walleriana*							
I-0201 x I-0103	pink purple	61C	scattered clumped	crenate	6-7	0	0
I-0201 x I-0104	magenta	57A	scattered clumped	serrate	8-10	2	2
I-0202 x I-0102	pink	52B	scattered clumped	-	10	1	1
I-0203 x I-0106	orange-red	40A	scattered clumped	crenate	5-6	1	0
I-0203 x I-0102	red	43A	scattered & clumped	crenate	6-7	0	0
I-0203 x I-0105	salmon	43C	scattered	crenate	5-6	0	0
I-0203 x I-0103	salmon	41C	scattered clumped	crenate	6	0	0

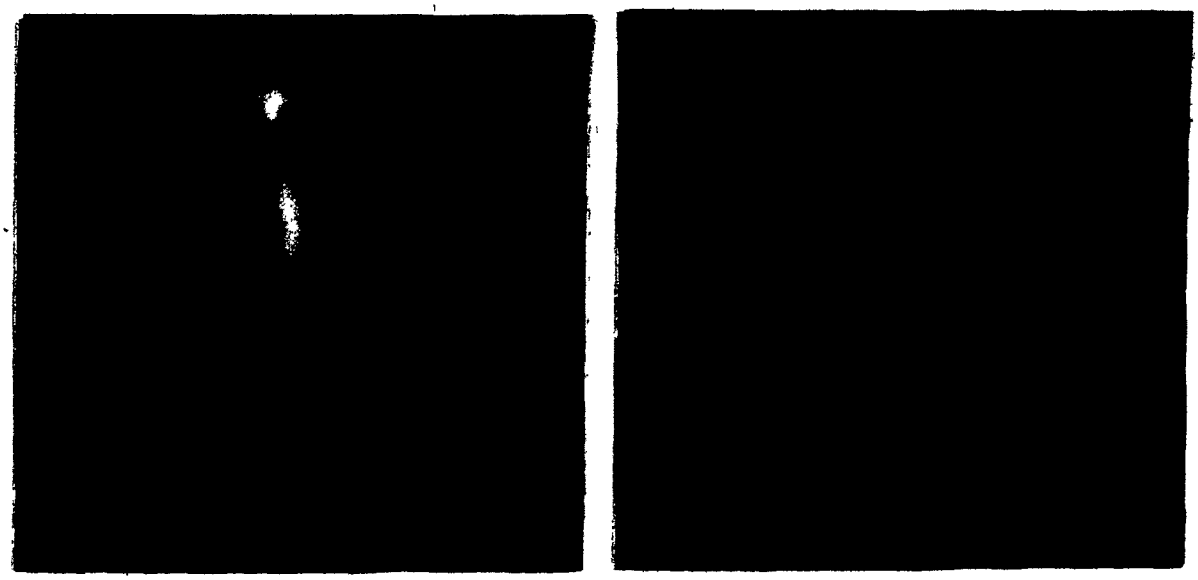
(table continued)

TABLE 4.3 (continued)

Accession number	Flower colour	RHS* flower colour number	Position of extrafloral nectaries	Leaf margin	No. pairs leaf lateral veins	Rank leaf pubescence**	
						top	bottom
I. walleriana x *I. gordonii*							
I-0103 x I-0301	pink	75B	scattered	crenate	6-7	0	0
I-0106 x I-0301	pink	75B	scattered	crenate	5-7	0	0
I. gordonii x *I. walleriana*							
I-0301 x I-0102	pink	58C	scattered	crenate	5-6	0	0
I-0301 x I-0104	pink	52B	scattered	crenate	5-6	0	0
I-0301 x I-0105	violet	66C	scattered	crenate	7-8	0	0
I-0301 x I-0111	lavender	75B & 57C	scattered	crenate	5-6	0	0

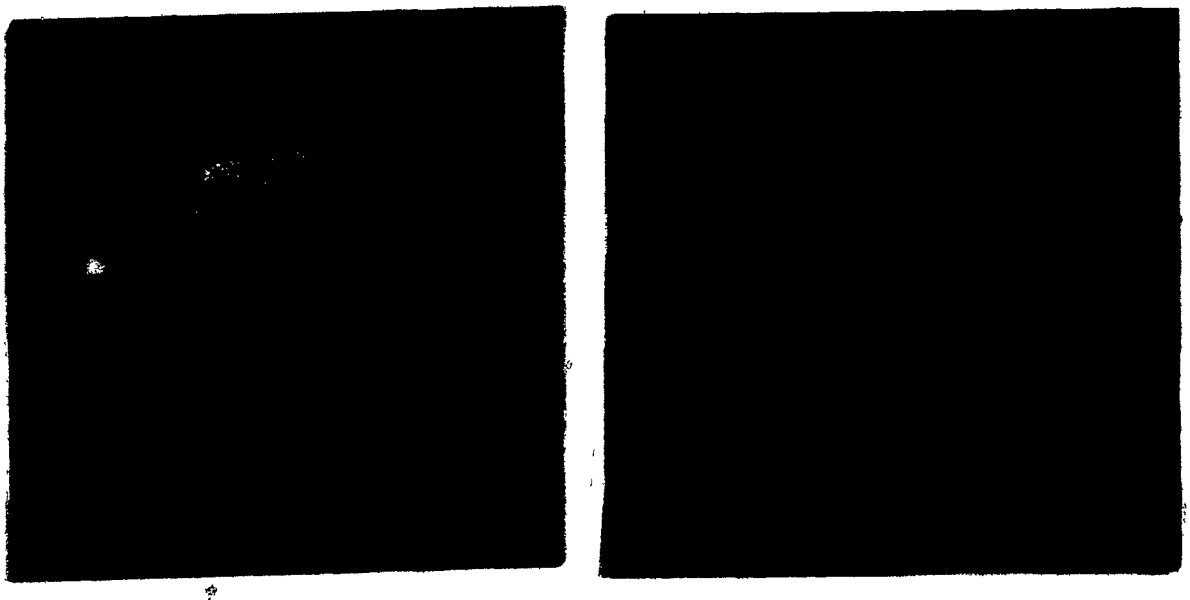
*RHS - Royal Horticultural Society

**Leaf pubescence ranks: 0=glabrous; 1=very slightly pubescent when seen under the dissecting microscope; 2=pubescent to the naked eye; 3=heavily pubescent.



(a) I-0201 x I-0104
I. usambarensis x I. walleriana

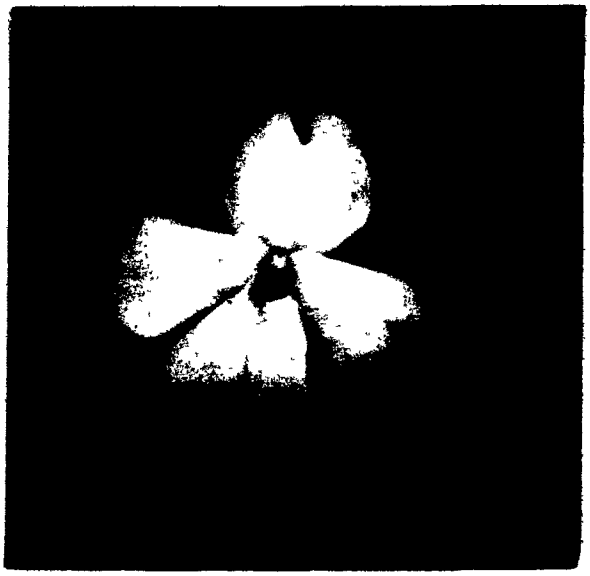
(b) I-0203 x I-0102
I. usambarensis x I. walleriana



(c) I-0202 x I-0102
I. usambarensis x I. walleriana

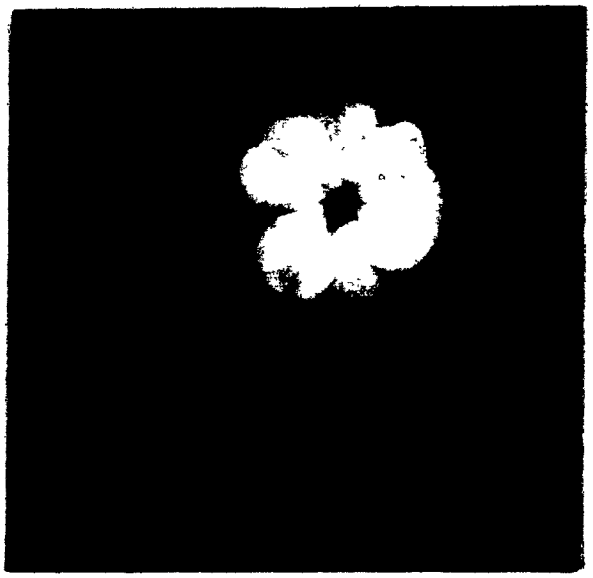
(d) I-0103 x I-0202
I. walleriana x I. usambarensis

Figure 4.5. Flowers of I. walleriana x I. usambarensis (and reciprocal) hybrids.



(a) I-0103 x I-0301

I. walleriana x I. gordonii



(b) I-0301 x I-0111

I. gordonii x I. walleriana

(c) I-0301 x I-0104

I. gordonii x I. walleriana

Figure 4.6. Flowers of I. walleriana x I. gordonii (and reciprocal) hybrids.

Chapter 5

CYTOLOGICAL INVESTIGATIONS OF THE PARENTS

A summary of the cytological investigations for plants of the different accessions appears in Table 5.1. The table is limited to species that successfully hybridized with other species, plus a few species that were of particular interest. Under ideal conditions, 200 cells per meiotic stage were examined for each bud, and the results from two flower buds were averaged. Unfortunately, not every bud had 200 cells in the desired stage and it was not always possible to find two buds in the same stage. Therefore, 75-250 cells were examined and the results from 1-3 buds were averaged.

Cells from late pachytene to metaphase I were grouped under the heading MI. Abnormalities included univalents and precocious separation of bivalents. AI-TI included cells from anaphase I to telophase I. Bridges and lagging chromosomes were recorded as abnormalities when found. Cells from anaphase II and telophase II were grouped under the heading AII-TII. Abnormalities included bridges, lagging chromosomes, and multipolar telophases. The quartet cells had micronuclei and rarely meganuclei as abnormalities. Unstained pollen was considered to be abnormal. A detailed description of each accession follows.

TABLE 5.1. Cytological investigations of selected *Impatiens* accessions

Accession number	Species	Percentage abnormalities				
		MI	AI-TI	AII-TII	Quartets	Pollen
I-0101	*I. walleriana*	0.0	0.0	-*	0.4	14.1
I-0102	*I. walleriana*	0.0	0.0	-	0.3	0.8
I-0103	*I. walleriana*	0.0	0.8	0.7	0.0	1.4
I-0104	*I. walleriana*	0.0	0.8	0.0	0.3	9.4
I-0105	*I. walleriana*	0.0	1.2	-	0.3	3.0
I-0106	*I. walleriana*	0.0	-	-	0.0	0.7
I-0107	*I. walleriana*	0.0	0.0	-	0.0	2.5
I-0111	*I. walleriana*	0.0	0.5	0.7	0.6	12.2
I-0201	*I. usambarensis*	-	0.0	0.0	0.6	37.8
I-0202	*I. usambarensis*	1.5	0.0	-	8.7	51.3
I-0203	*I. usambarensis*	0.0	0.0	-	0.7	2.7
I-0301	*I. gordonii*	0.0	1.0	-	0.5	58.6
I-0401	*I. cinnabarina*	-	-	-	0.4	0.1
I-1001	*I. platypetala* var. *aurantiaca*	35.9	0.0	-	14.3	7.8
I-0802	*I. flaccida*	0.0	0.0	0.0	0.3	1.4
I-0801	*I. flaccida* var. *alba*	0.6	22.9	14.5	25.0	65.4
I-0801 x I-0802	*I. flaccida* (cross)	0.0	0.4	0.2	2.3	1.4
I-0801 x I-0801	*I. flaccida* var. *alba* (selfed)	-	-	-	-	43.0

* - = stage not obtained

Impatiens walleriana

Meiosis was normal, with the exception of one quartet cell in I-0101. Eight bivalents were formed in metaphase I in all cases. One micronucleus was observed in a quartet cell, and 4.1% of the pollen was unstained. The pollen was 4-colpate rectangular.

I-0102 also had regular meiosis except for one quartet cell. Eight bivalents were formed at metaphase I, and one quartet cell had fused pollen. Normal pollen was 4-colpate rectangular, and 0.8% was unstained.

In I-0103, meiosis was regular with the exception of a few cells. Metaphase I had 8 bivalents present in all cases. In anaphase I, two cells had one lagging univalent, and one cell had three lagging univalents. There was a pentapolar cell in telophase II, but no abnormal quartets were observed. Pollen was 4-colpate rectangular, and only 1.4% was unstained.

Only three cells were abnormal in I-0104. Eight bivalents were formed at mataphase I. One cell had lagging chromosomes in anaphase I, and two of the quartet cells had one micronucleus each. Pollen was 4-colpate rectangular, and 9.4% was unstained.

Meiosis was normal in I-0105, except for three cells. Eight bivalents were formed in metaphase I. Two anaphase I cells had three lagging univalents, and one of the quartet cells had five nuclei instead of four. Pollen was 4-colpate rectangular, and 3.0% was unstained.

No abnormalities were observed in the meiosis of I-0106. Metaphase I was normal with the expected 8 bivalents. All quartet cells were normal, and only 0.7% of the pollen was unstained. The pollen was 4-colpate rectangular.

No meiotic abnormalities were observed in I-0107. Eight bivalents were formed at metaphase I, and anaphase I and the quartet stages were regular. The pollen was 4-colpate rectangular, and only 2.5% was unstained.

There were more meiotic abnormalities in I-0111. Metaphase I was normal with eight bivalents formed in all cases. However, chromosome bridges were seen in one cell in anaphase I, and one cell in telophase II. Three quartet cells had one micronucleus, and one cell had two micronuclei. The pollen was 4-colpate rectangular and 12.2% was unstained.

Impatiens usambarensis

Meiosis was normal in I-0201, except for the quartets in which one cell had one micronucleus, and two cells had two micronuclei. Anaphase I and telophase II were completely normal. Pollen was 4-colpate rectangular, and a large proportion (37.8%) was unstained. Unfortunately, metaphase I was never observed in the 50-60 buds which were examined.

I-0202 had a more irregular meiosis. With the exception of four cells, metaphase I was normal with 8 bivalents. However, two metaphase I cells had seven bivalents at the equator, and a univalent at either

pole. Another two cells had seven bivalents at the equator, and two univalents at one of the poles. Anaphase I was completely normal. The quartet cells ranged from a high proportion of abnormalities (25.6%) on one day to no abnormalities on another day in different buds from the same plant. On a third day, 4.6% of the quartet cells were abnormal. The abnormal quartets had 1-5 extra micronuclei. Pollen was 4-colpate rectangular, and over half was unstained (51.3%).

With the exception of one quartet cell, meiosis was regular in I-0203. Eight bivalents were formed in metaphase I, and anaphase I was normal. One quartet cell had one micronucleus. Pollen was 4-colpate rectangular, and a small proportion (2.7%) was unstained.

Impatiens gordonii

Meiosis was regular in I-0301, with the exception of a few cells. Metaphase I had eight bivalents present in all cases. Anaphase I was normal except for one cell that had a lagging chromosome. Two quartet cells had one micronucleus. The pollen was 4-colpate rectangular, and over half (58.6%) was unstained.

Impatiens cinnabarina

The early stages of meiosis were not observed in I-0401, but the quartet cells were usually normal. One cell had one micronucleus, and another cell had two micronuclei. Pollen was 4-colpate rectangular with a very small proportion (0.1%) unstained.

Impatiens platypetala var. aurantiaca

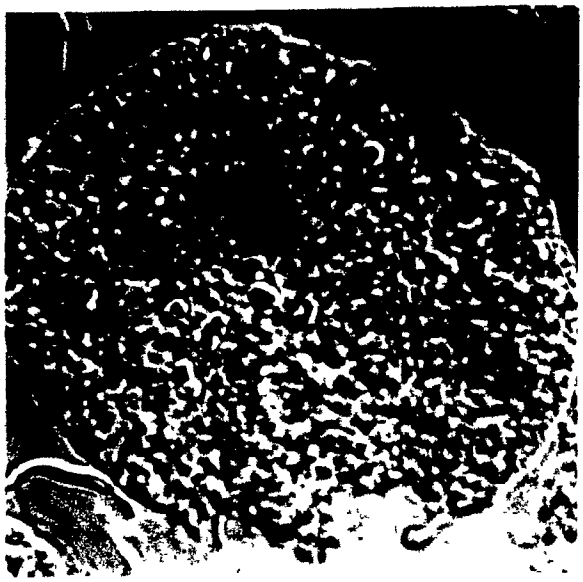
Meiosis in I-1001 was highly irregular, with a high proportion (35.9%) of metaphase I cells with univalents. The chromosomes in metaphase I were extremely small, but with the use of Nomarsky interference contrast optics they could be differentiated. In one bud, 25 cells had 7 bivalents, 13 cells had 6 bivalents and 2 univalents (Figure 5.1a), and 1 cell had 5 bivalents and 4 univalents.

There was also a high degree (14.3%) of abnormality in the quartets. Cells had from one to ten micronuclei. Some cells had two meganuclei instead of four normal nuclei (Figure 5.1b). However, there was a fairly low proportion of unstained pollen (7.8%) when all the meiotic abnormalities were taken into account. Pollen had 2, 4, or 5 pores, with 4-colpate square pollen probably being the norm.

Impatiens flaccida

Meiosis was slightly irregular in I-0802. There were usually 7 bivalents formed in metaphase I, but some cells were found with 14 univalents. Anaphase I and telophase II were normal. One quartet cell with a tiny micronucleus was also observed. The pollen was 3-colpate triangular, and a very small proportion was unstained (1.4%).

Meiosis was very irregular in I-0801 (I. flaccida var. alba). Seven bivalents were usually formed in metaphase I, but one cell had 6 bivalents and 2 univalents. There was a high proportion of lagging chromosomes in anaphase I. Chromosomes sometimes remained at the equator in telophase I. Abnormalities of telophase II included lagging



(a) Metaphase I: 6 II's + 2 I's
Mag. X 1094

(b) Abnormal quartets
Mag. X 438

Figure 5.1. Meiotic abnormalities of I. platypetala var. aurantiaca (I-1001).

chromosomes and multipolar arrangements of chromosomes. One-quarter of the quartet cells were abnormal with one to seven micronuclei. Pollen was 3-colpate triangular, and over half (65.4%) was unstained. A selfed cross of I-0801 also had a high proportion of unstained pollen (43.0%).

The cross between I-0801 and I-0802, on the other hand, had a low proportion of unstained pollen (1.4%). Meiosis in I-0801 x I-0802 was regular with the exception of a few cells. Eight bivalents were formed at metaphase I in all cases. One telophase I cell had a lagging chromosome, and one telophase II cell was pentapolar. Micronuclei were observed in 35 quartet cells, but only 2.3% were abnormal.

Chapter 6

CYTOLOGICAL INVESTIGATIONS OF THE HYBRIDS

A summary of the cytological investigations of the hybrids is given in Table 6.1 for I. walleriana x I. usambarensis, and Table 6.2 for I. walleriana x I. gordonii. The examination of the buds was performed as described for the parents in the initial paragraphs of Chapter 5. The hybrid plants derived from the species crosses will be discussed separately.

Impatiens walleriana x I. usambarensis

Meiosis in the I. walleriana x I. usambarensis (and in their reciprocal) hybrids, with the exception of some occasional irregularities given below, was normal until the quartet stage, where most of the abnormalities occurred. Very occasionally, univalents were seen at metaphase I, but in most cases, 8 bivalents were formed. Bridges and lagging chromosomes were sometimes seen in anaphase I and telophase I. A few abnormalities were observed at telophase II. These included bridges, lagging chromosomes, and multipolar arrangements of chromosomes. Pollen was 4-colpate rectangular with a low proportion unstained except for that from one hybrid. Another hybrid did not produce any pollen. However, it must be stressed that in the vast majority of cells, meiosis was completely regular. A detailed discussion of the individual I. walleriana x I. usambarensis hybrids follows.

TABLE 6.1. Cytological investigations of *I. walleriana* x *I. usambarensis* and reciprocal hybrids

Cross	Percentage abnormalities				
	MI	AI-TI	AII-TII	Quartets	Pollen
I. walleriana x *I. usambarensis*					
I-0101 x I-0201A	0.0	0.0	0.0	100.0*	100.0*
I-0101 x I-0201B	0.0	0.0	0.0	100.0*	100.0*
I-0101 x I-0201C	1.6	1.3	12.5	100.0*	100.0*
I-0103 x I-0202A	0.0	0.4	0.0	0.0	0.7
I-0106 x I-0201A	-**	-	-	0.9	3.9
I. usambarensis x *I. walleriana*					
I-0201 x I-0103A	0.0	1.3	-	1.4	2.8
I-0201 x I-0103B	-	-	-	1.2	3.7
I-0201 x I-0104A	0.0	0.0	0.0	1.5	1.9
I-0201 x I-0104B	-	-	-	0.6	1.8
I-0201 x I-0104C	1.8	6.0	0.0	0.0	1.4
I-0201 x I-0104D	0.0	0.0	-	0.0	1.1
I-0202 x I-0102A	-	-	-	0.0	44.4
I-0203 x I-0106A	-	-	-	1.5	6.8
I-0203 x I-0106B	6.7	2.8	0.0	0.5	-
I-0203 x I-0106C	-	-	-	-	-
I-0203 x I-0105A	-	-	-	0.0	1.9
I-0203 x I-0102A	-	-	-	-	4.8
I-0203 x I-0103A	0.0	3.3	1.4	1.0	2.5
I-0203 x I-0103B	-	0.0	0.0	3.6	-
I-0203 x I-0103C	0.0	-	-	-	-

*Considered 100% abnormal because quartets and pollen never formed.

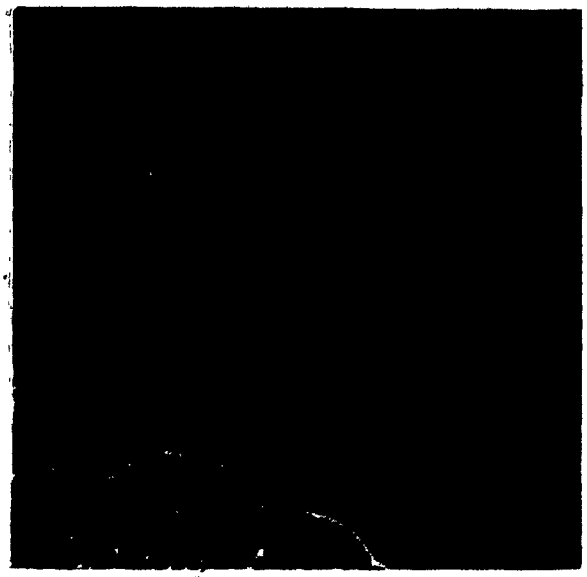
** - = stage never observed.

I-0101 x I-0201.--With the exception of a few cells, meiosis was normal in I-0101 x I-0201 until the quartet stage. Eight bivalents were observed at metaphase I in all cases for two of the hybrids (I-0101 x I-0201 A, B) (Figure 6.1a). The third hybrid (I-0101 x I-0201C) had 9 cells with 7 bivalents and 2 univalents. Meiosis was completely regular in the first two hybrids until late telophase II. In the third hybrid, there were 8 cells with a bridge in anaphase I; and telophase II had one cell with a penta-polar arrangement of chromosomes, and one cell with lagging chromosomes. In all three hybrids, mature quartets were never formed. There is some evidence that quartets started to form and then disintegrated (Figure 6.1b). No pollen was ever produced.

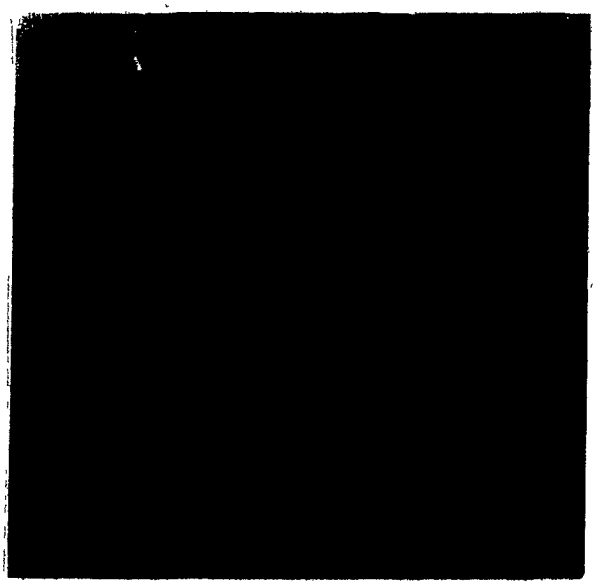
I-0103 x I-0202.--Only one meiotic cell was observed as irregular in I-0103 x I-0202. In all cases, eight bivalents were formed at metaphase I. One anaphase I cell had a lagging univalent. Telophase II and the quartet cells were all normal. Pollen was 4-colpate rectangular, and only 0.7% was unstained.

I-0106 x I-0201.--The quartet cells were normal in I-0106 x I-0201, except for two cells which had an extra micronucleus. Pollen was 4-colpate rectangular, and 3.9% was unstained. Inexplicably this plant disappeared from the greenhouse before more data could be collected.

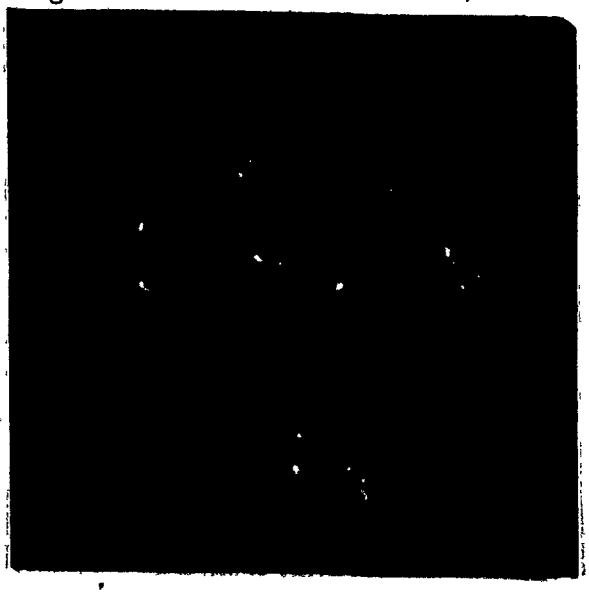
I-0201 x I-0103.--Meiosis was regular in I-0201 x I-0103, with the exception of a few cells. Eight bivalents were observed at metaphase I, and two anaphase I cells had a bridge. In the first hybrid (I-0201 x



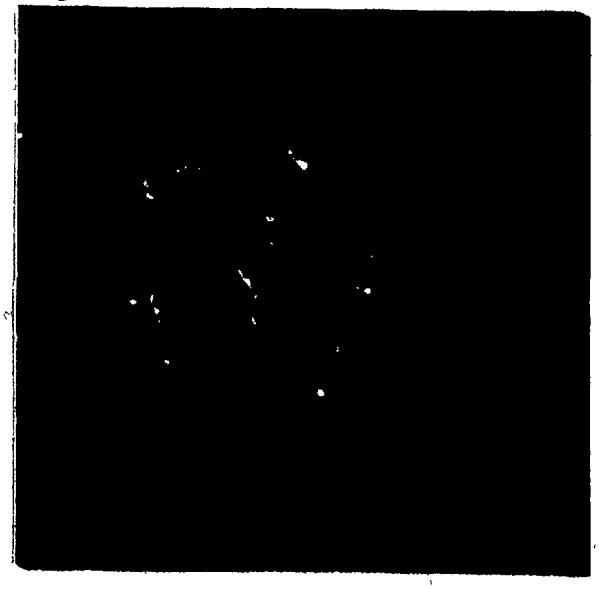
(a) I-0101 x I-0201
I. walleriana x I. usambarensis
Metaphase I: 8 II's
Mag. X 381



(b) I-0101 x I-0201
I. walleriana x I. usambarensis
Disintegrating quartets
Mag. X 569



(c) I-0203 x I-0106
I. walleriana x I. usambarensis
Metaphase I: 7 II's + 2 I's
Mag. X 1000



(d) I-0203 x I-0106
I. walleriana x I. usambarensis
Metaphase I: 6 II's + 4 I's
Mag. X 1000

Figure 6.1. Meiotic cells of I. walleriana x I. usambarensis and reciprocal hybrids.

I-0103A), three quartet cells had an extra micronucleus, and one cell had fused pollen. Pollen was 4-colpate rectangular, and 3.8% was unstained.

I-0201 x I-0104.--In I-0201 x I-0104, meiosis was regular except for a few cells. Five cells of the third plant (I-0201 x I-0104C) had 7 bivalents and 2 univalents, but all other metaphase I cells had the normal 8 bivalents. Anaphase I was normal except for 4 cells with lagging chromosomes in the third hybrid. No irregularities were noted in telophase II. Pollen was 4-colpate rectangular and an average of 1.6% was unstained. The fourth hybrid (I-0201 x I-0104D) had a flower colour which differed from the other three, and the pollen was much squarer than that of the other three plants.

I-0202 x I-0102.--No abnormalities of the quartet cells were observed in I-0202 x I-0102. However, a large proportion (44.4%) of the pollen was unstained. The pollen was usually 4-colpate rectangular, but the occasional grain had 5 pores, and sometimes two pollen grains were fused together. Unfortunately, the early stages of meiosis were never obtained for study.

I-0203 x I-0106.--Meiosis was slightly irregular in I-0203 x I-0106. The expected 8 bivalents were found at metaphase I most of the time. However, 13 cells had 7 bivalents and 2 univalents; and 2 cells had 6 bivalents and 4 univalents (Figure 6.1c,d). One anaphase I cell had a lagging univalent, but telophase II was normal. The quartet cells sometimes had extra micronuclei: 15 had 1 extra micronucleus,

5 had 2 extra, and 1 had 3 extra. Pollen was 4-colpate rectangular, with a low percentage (6.8%) unstained.

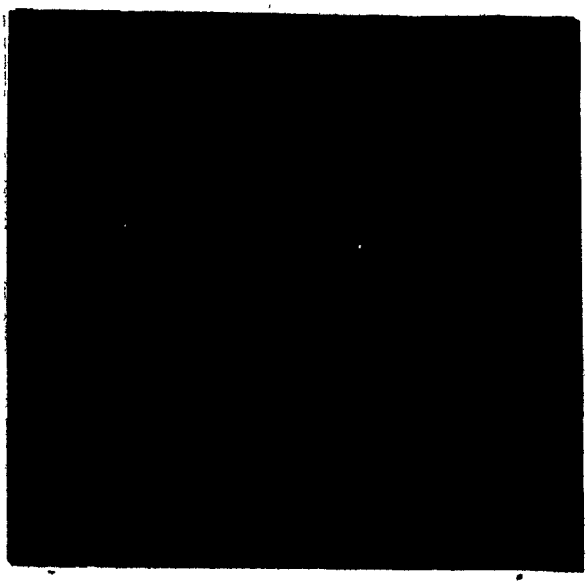
I-0203 x I-0105.--The quartet cells were completely normal in I-0203 x I-0105. Pollen was 4-colpate rectangular, and a low proportion (1.9%) was unstained. The early stages of meiosis were never found, even though 75-90 buds were examined.

I-0[illegible] x I-0102.--I-0203 x I-0102 had 4-colpate rectangular pollen and 4.8% was unstained. This plant produced only a few flowers, and the few buds gathered were either too early or too late for the observation of meiotic stages.

I-0203 x I-0103.--With the exception of a few cells, meiosis was normal in I-0203 x I-0103. Eight bivalents were formed at metaphase I. The first hybrid (I-0203 x I-0103A) had four cells with one or two lagging chromosomes at anaphase I, and one anaphase II cell with two lagging univalents (Figure 6.2a). Anaphase I and telophase II were regular in the second hybrid (I-0203 x I-0103B). Two quartet cells had an extra micronucleus in the first hybrid; and 19 had 1 extra micronucleus, 5 had 2 extra, and 1 had 4 extra in the second hybrid. Pollen was 4-colpate rectangular, and 2.5% was unstained.

Impatiens walleriana x I. gordonii

Meiosis in the I. walleriana x I. gordonii and their reciprocal hybrids was usually regular. Eight bivalents were almost always formed at metaphase I, but one hybrid had from 0 to 8 univalents. Anaphase I

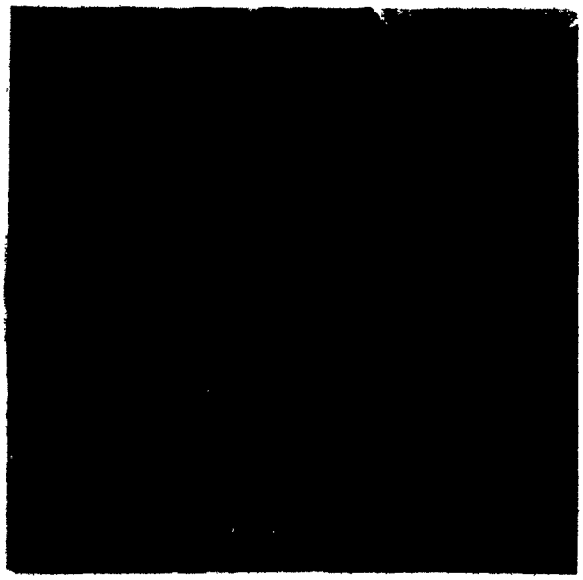


(a) I-0203 x I-0103

I. usambarensis x I. walleriana

Anaphase II: lagging chromosomes

Mag. X 612



(b) I-0103 x I-0301

I. walleriana x I. gordonii

Anaphase I: bridge

Mag. X 447

Figure 6.2. Meiotic anaphase cells in Impatiens hybrids.

TABLE 6.2. Cytological investigations of *I. walleriana* x *I. gordonii* and reciprocal hybrids

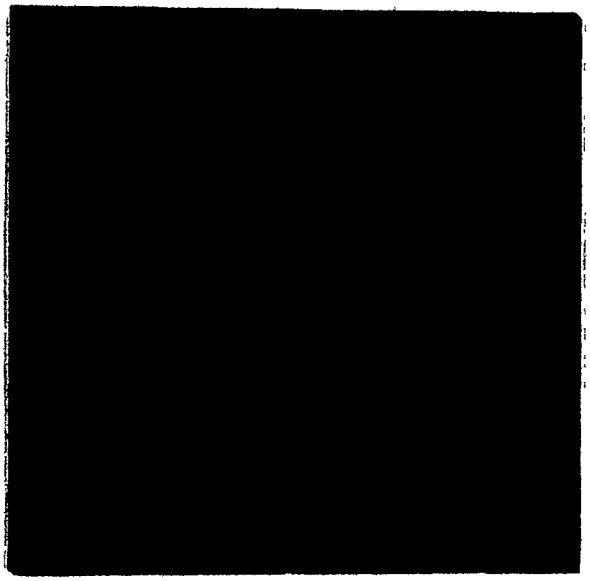
Cross	Percentage abnormalities				
	MI	AI-TI	AII-TII	Quartets	Pollen
I. walleriana x *I. gordonii*					
I-0103 x I-0301A	3.6	0.5	0.0	0.5	22.9
I-0103 x I-0301B	15.5	0.0	-*	41.7	47.5
I-0106 x I-0301A	-	-	-	-	-
I-0106 x I-0301B	-	-	-	0.4	48.7
I-0106 x I-0301C	0.0	-	-	-	17.5
I. gordonii x *I. walleriana*					
I-0301 x I-0102A	-	-	-	-	33.1
I-0301 x I-0102B	-	3.8	0.0	0.8	-
I-0301 x I-0102C	-	-	-	7.5	-
I-0301 x I-0104A	-	0.0	0.0	0.7	24.1
I-0301 x I-0104B	0.0	0.0	0.0	0.9	28.8
I-0301 x I-0104C	0.0	-	-	3.8	30.4
I-0301 x I-0105A	-	-	-	20.5	-
I-0301 x I-0105B	-	-	-	1.5	64.8
I-0301 x I-0105C	-	-	-	-	-
I-0301 x I-0111A	-	-	-	-	-
I-0301 x I-0111B	0.0	4.5	-	1.4	58.3

* - = stage not observed

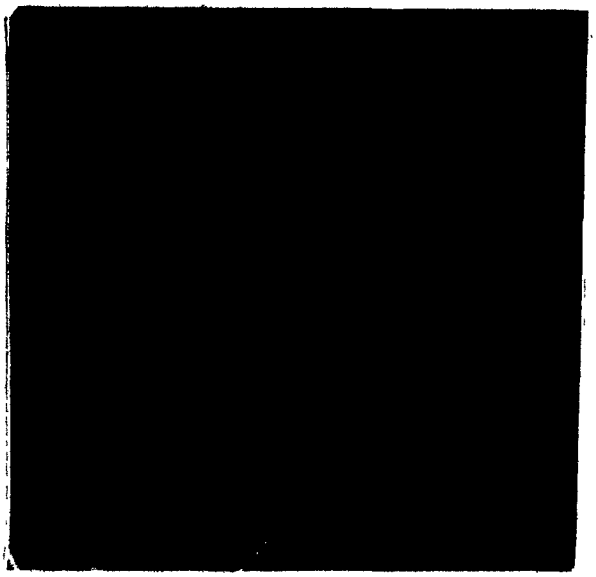
abnormalities included bridges and lagging chromosomes. Telophase II was normal. Abnormalities of the quartet cells included extra micronuclei, unfilled pollen, and unusual-shaped pollen. Pollen was 4-colpate rectangular, and a high proportion (23-65%) was unstained. Unfortunately, many meiotic stages were never examined due to an insufficient number of buds obtained. The I. gordonii x I. walleriana hybrids did not produce very many flowers, and a number of the hybrids became infected with a virus and stopped flowering completely.

I-0103 x I-0301.--Meiosis was somewhat irregular in I-0103 x I-0301. Most cells had eight bivalents at metaphase I, but 4 cells had 7 bivalents and 2 univalents in the first hybrid (I-0103 x I-0301A). More univalents were seen in the second hybrid (I-0103 x I-0301B): 6 cells had 7 bivalents and 2 univalents, 2 cells had 6 bivalents and 4 univalents, and 1 cell had 4 bivalents and 8 univalents. Only one cell had a bridge in anaphase I (Figure 6.2b). Telophase II was normal. In the first hybrid the quartet cells were normal with the exception of one cell in which there was an extra micronucleus. However, the quartets were very unusual in the second hybrid and 35-48% were abnormal. Abnormalities included from one to many micronuclei, amorphous blobs, amorphous blobs with micronuclei, and fused pollen (Figure 6.3d). Pollen was 4-colpate rectangular, and 22.9% was unstained in the first hybrid, and 47.5% was unstained in the second.

I-0106 x I-0301.--With the exception of one quartet cell, meiosis was regular in I-0106 x I-0301. The usual 8 bivalents were formed at metaphase I (Figure 6.3a). One quartet cell had 2 micronuclei; the



(a) I-0106 x I-0301
I. walleriana x I. gordonii
Metaphase I: 8 II's
Mag. X 715



(b) I-0301 x I-0105
I. gordonii x I. walleriana
Abnormal quartet
Mag. X 381

(c) I-0301 x I-0104
I. gordonii x I. walleriana
Abnormal quartet
Mag. X 720

(d) I-0103 x I-0301
I. walleriana x I. gordonii
Abnormal quartet
Mag. X 452

Figure 6.3. Meiotic cells of I. walleriana x I. gordonii and reciprocal hybrids.

rest were normal. I-0106 x I-0301 produced very few flowers, so there were insufficient buds for an examination of the other stages of meiosis. Pollen was 4-colpate rectangular, and 17.5-48.7% was unstained.

I-0301 x I-0102.--I-0301 x I-0102 had regular meiosis with the exception of a few cells. Three anaphase I cells had lagging chromosomes in the second hybrid (I-0301 x I-0102B), but telophase II was normal. Fifteen quartet cells had 1 micronucleus, 3 had 2 micronuclei, and 1 had 3 micronuclei. Pollen was 4-colpate rectangular and 33% was unstained. Unfortunately, these plants became infected by a virus and stopped flowering before metaphase I could be observed.

I-0301 x I-0104.--Meiosis was normal in the I-0301 x I-0104 hybrids except for a few quartet cells. Eight bivalents were formed at metaphase I in all cases. Anaphase I and telophase II were normal in the first two hybrids (I-0301 x I-0104A, B). Twenty-two quartet cells had 1 extra micronucleus, 10 cells had 2, and 1 cell had 4 (Figure 6.3c). Pollen was 4-colpate rectangular and 24-30% was unstained.

I-0301 x I-0105.--The quartet cells in I-0301 x I-0105 were rather irregular, especially in the first hybrid (I-0301 x I-105A) which had 17-23% of the quartets with extra micronuclei, and one very unusual cell (Figure 6.3b). The second hybrid (I-0301 x I-0105B) had only three cells with extra micronuclei. In this hybrid, pollen was 4-colpate rectangular, and 64.8% was unstained. It would have been very interesting to have observed the early stages of meiosis in this hybrid, but these plants were not prolific flowerers, so the other stages of meiosis were never seen.

I-0301 x I-0111.--In I-0301 x I-0111, meiosis was regular except for the occasional cell. As usual, 8 bivalents were formed at metaphase I; but one cell had a bridge at anaphase I. Five quartet cells had an extra micronucleus, and 3 cells had 2 micronuclei. Pollen was 4-colpate rectangular, and 58.3% was unstained. The first hybrid became infected with a virus and never flowered, so all data were taken on the second hybrid (I-0301 x I-0111B).

Comparison of the two types of hybrids

In conclusion, meiosis was slightly more irregular in I. walleriana x I. gordonii than in I. walleriana x I. usambarensis. Univalents were seen at metaphase I in 3 out of 6 I. walleriana x I. usambarensis hybrids, and in 1 out of 4 I. walleriana x I. gordonii hybrids. With the exception of two cells with 6 bivalents and 4 univalents, I. walleriana x I. usambarensis had 7 bivalents and 2 univalents in all cells where the normal 8 bivalents were not formed. I. walleriana x I. gordonii, on the other hand, had one cell with 4 bivalents and 8 univalents.

The median test was used to determine whether I. walleriana x I. gordonii had a higher percentage of abnormal quartets or unstained pollen than I. walleriana x I. usambarensis. In both cases, the former had a significantly higher number of abnormalities at the 0.05 level of confidence ($p = 0.035$). However, it must be stressed once again that even though there were occasional abnormalities in both hybrids, meiosis was perfectly normal in the vast majority of cells.

Chapter 7

DISCUSSION

The results of the crossing experiments were very similar to those of by Arisumi (1980a). Of the species studied in common, only I. walleriana and I. gordonii hybridized in both cases. The species which failed to cross in Arisumi's study also failed to hybridize in this one. However, Arisumi obtained a hybrid between I. sodenii and I. flaccida by using ovule-culture techniques (1980b). It would be very interesting to examine the meiotic configurations in this hybrid because I. sodenii is of African origin and I. flaccida is of Indonesian origin. It is quite possible that other hybrids may be produced by the ovule-culture technique of Arisumi (1980b).

Arisumi (1980a) did not use I. usambarensis in his study, but Grey-Wilson (1980a, 1980d) described putative natural hybrids between I. walleriana and I. usambarensis. The artificial hybrids obtained in this study help confirm his field observation that the two species hybridize.

Meiosis in both the I. walleriana x I. gordonii, and I. walleriana x I. usambarensis hybrids was normal in almost all cases, with the occasional abnormality occurring usually in the later stages of meiosis. Since two of the species in the study had unusual meiosis, they will be

discussed first so that meiotic abnormalities of the hybrids can be considered in light of those occurring within Impatiens species.

There was an unexpectedly high number of univalents at metaphase I in I. platypetala var. aurantiaca, and this could be the reason for the abnormalities observed in the later stages of meiosis. However, the low percentage of unstained pollen compared with the percentage of abnormal quartets is puzzling because the opposite is usually true in Impatiens. It is possible that pollen from abnormal quartets may still be fertile even though it is not necessarily carrying the normal haploid complement of chromosomes. Such pollen grains could lead to dysploidy within the same species, as suggested by Zinov'eva-Stahevitch and Grant (1985b). Dysploidy is known in I. platypetala and I. platypetala var. aurantiaca (Zinov'eva-Stahevitch and Grant 1985a). Also, if a pollen grain united with an ovule with a different chromosome complement, one might expect to find univalents at metaphase I in the offspring. It would be very rewarding to investigate this species in greater depth.

The high proportion of quartet abnormalities and unstained pollen observed in I. flaccida var. alba (I-0801) could have a genetic basis because crosses between I-0801 x I-0802 produced plants that had very few quartet abnormalities and a low proportion of unstained pollen; and selfed plants of I-0801 retained a high degree of unstained pollen.

Both the I. walleriana x I. gordonii and I. walleriana x I. usambarensis hybrids, on the other hand, usually had regular meiosis leading to at least 50% fertile pollen in all but one case. All hybrids

had eight bivalents formed in the vast majority of metaphase I cells, indicating that both I. usambarensis and I. gordonii are closely related to I. walleriana. The I. gordonii x I. walleriana hybrids had the highest number of univalents in all the hybrids, i.e., 8 vs. 4; and also had a high proportion of quartet abnormalities, with more unusual quartets than I. usambarensis x I. walleriana. However, the I. walleriana x I. usambarensis cross (I-0101 x I-0201) was the only completely sterile hybrid.

In I-0101 x I-0201, mature quartets were never formed, and all three hybrids were completely sterile. As mentioned in Chapter 2, complete male sterility does occur in I. walleriana. Because normal quartets were never observed in I-0101 x I-0201, the male sterility could be similar to that reported by Tara and Namboordiri (1976) where sterility was due to a defect in cytoplasmic furrowing at the quartet stage. However, pollen walls were formed in I. walleriana (Tara and Namboordiri 1976), whereas they were not formed in this case.

On the other hand, the percentage of unstained pollen in the I. walleriana x I. gordonii hybrids was significantly higher than that of the I. walleriana x I. usambarensis hybrids. However, it was also much higher than one would expect from the percentage of quartet abnormalities (Table 6.2). I. gordonii also had a low percentage of quartet abnormalities compared with the high proportion of unstained pollen (Table 5.1). This phenomenon also occurs in the I. usambarensis accession I-0202 (Table 5.1), and the I. usambarensis x I. walleriana hybrid I-0202 x I-0102 (Table 6.1). In these cases, sterile pollen could be due to genetic factors and not the cytological factors

suggested by Zinov'eva-Stahevitch and Grant (1985b). If this is true, the percentage sterile pollen may not be a good character for evaluating differences between the two types of hybrids.

On the whole, meiotic abnormalities in the two types of hybrids were quite similar to irregularities found within Impatiens species, particularly I. flaccida var. alba, where the abnormalities may have a genetic origin. The only abnormalities not seen within the original accessions were the unusual quartets found in some I. walleriana x I. gordonii hybrids (Figure 6.3). The similarity of meiotic abnormalities in hybrids and the original species accessions could be because many Impatiens species are believed to be of hybrid origin, including I. walleriana and I. cinnabarina (Grey-Wilson 1980a), and therefore, the irregularities observed within a species may be a relict of their hybrid origin.

Nakamura (1935) found that meiotic abnormalities in I. balsamina were caused by air temperatures of 30°C and above. Abnormalities included univalents at metaphase I, lagging chromosomes at anaphase I, and chromosomes organizing into more than four groups at anaphase II. Because many of the flower buds in this study were collected during the summer, when the greenhouse temperature often rose above 30°C, it is possible that the abnormalities observed could be a manifestation of high temperatures. However, the buds collected from the I. walleriana accessions, I-0102, I-0103, I-0104, I-0105, I-0106, and I-0107, and the I. usambarensis accessions, I-0201 and I-0203, on the hottest collection day of the year showed almost no abnormalities. Since the hybrids which had meiotic abnormalities were collected under much

cooler conditions, it is quite possible that the meiotic abnormalities are not the result of high temperatures.

The final possibility is that I. gordonii and I. usambarensis are not valid species, but part of the natural variation of I. walleriana. With this in mind, the status of the two taxa will now be considered.

Impatiens gordonii can easily be distinguished from I. walleriana on a morphological basis. The leaves are a different colour, shape and size from those of I. walleriana. The flower is about the size of the commercial cultivar I. walleriana cv. 'Blitz,' but much larger than the wild accessions. Because of the clear-cut morphological differences and the greater number of meiotic abnormalities in the hybrid, I feel that I. walleriana and I. gordonii are two distinct, but closely allied species.

It is interesting to speculate why two very similar species are found such a great distance apart. The Seychelles were once part of Gondwanaland, but both I. walleriana and I. gordonii are considered to be of recent origin (Grey-Wilson 1978), so their recent progenitors would not have been present at the breakup of Gondwanaland. Recorded colonization of the Seychelles did not occur until the 18th century, and even then it was used as a penal colony by French Mauritius on the west coast of Africa where I. walleriana does not grow. However, the islands may have been visited by marauding pirates or fishermen from Zanzibar (where I. walleriana does occur) before this time.

Separation of I. walleriana and I. usambarensis on a morphological basis is a little more difficult. The five characters in Table 2.1

(after Grey-Wilson 1980a) are not very useful to distinguish the species. From Table 5.2, one can see that all I. usambarensis accessions have a pubescent leaf lamina, but so do three I. walleriana accessions: I-0101, I-0104, and I-0111. All I. walleriana accessions have leaves with crenate margins, while two I. usambarensis accessions (I-0201 and I-0203) have some leaves with serrate margins and some leaves with crenate margins. The number of leaf lateral veins is not a good character for separating the two taxa because I. walleriana has from 5 to 9 pairs, and I. usambarensis has from 6 to 8 pairs.

Of all the characters in Table 2.1, the position of the leaf petiole glands seems to be the best for separating the two taxa. I. usambarensis always has clumped extra-floral nectaries, and in all but one case I. walleriana has extra-floral nectaries which are scattered. The I. walleriana accession I-0107 has scattered clumps of extra-floral nectaries, a configuration found to be characteristic of I. walleriana x I. usambarensis hybrids (Grey-Wilson 1980a).

Flower measurements were not taken because many of the I. walleriana accessions became infected with a virus which reduced flower size. Because there is a great deal of overlap in the lengths of the lateral united petals between I. walleriana and I. usambarensis (Table 2.1) the length of lateral united petals may not be a useful character for the separation of the two taxa, and neither are leaf pubescence, leaf margins, or the number of leaf lateral veins.

The three I. usambarensis accessions had flowers with crispate petal margins. I. walleriana flowers almost always had petals with

entire margins, but some of the I. walleriana x I. walleriana progeny had petals with slightly crispate margins.

There may be a cytological character that will differentiate I. walleriana and I. usambarensis. All three I. usambarensis accessions have one pair of chromosomes with satellites, whereas satellites have been observed in only one I. walleriana accession. Likewise, there is only one report of satellites in I. walleriana in the literature (Smith 1934). Since there was no mention of where the plant in Smith's study originated, and because no herbarium voucher was ever deposited, the possibility that this plant may have been I. usambarensis cannot be ruled out. The I. walleriana (I-0108) in our collection having one pair of satellites has glabrous leaves with crenate margins and clumped extra-floral nectaries. It also has flowers with crispate petal margins reminiscent of I. usambarensis. We are waiting for Dr. C. Grey-Wilson of the Royal Botanic Gardens, Kew, England, to give us his opinion on whether or not this accession could be I. usambarensis, as it is possible that this plant was misidentified or mislabelled at Kew.

Therefore, the status of I. usambarensis is less certain than that of I. gordonii. I. walleriana is not native to the Usambara Mountains where I. usambarensis is endemic (Grey-Wilson 1980a). It is possible that a few I. walleriana plants became isolated on the Usambara Mountains by climatic conditions described by Grey-Wilson (1980a; see Chapter 2), and by interbreeding produced the entity known as I. usambarensis. The re-introduction of I. walleriana to the area has made hybridization possible (Grey-Wilson 1980a). Because I. walleriana forms fertile hybrids with I. usambarensis, the taxon may not be able to

maintain its genetic integrity and it could become re-absorbed into the I. walleriana gene pool.

However, I. usambarensis is a forest species found growing in moist shady and semi-shady areas and I. walleriana prefers more open sites. The hybrid is usually associated with disturbed habitats (Grey-Wilson 1980a), so perhaps I. usambarensis is an ecological species derived from I. walleriana. I have also noticed that I. usambarensis has a tendency to stop flowering when the days become very short in December and January, but I. walleriana does not.

Impatiens gordonii, on the other hand is in no danger of losing its genetic identity because I. walleriana does not grow in the Seychelles. However, if I. walleriana does become naturalized to the Seychelles, I. gordonii could be endangered because Arisumi (1980a) has found that the hybrid between I. gordonii and I. walleriana can be successfully back-crossed with I. walleriana, but not with I. gordonii. This could put I. gordonii at a severe disadvantage.

A hybrid between I. usambarensis and I. gordonii was never obtained, even though three crosses were attempted. Because I. gordonii hybridizes readily with I. walleriana, one would expect that it would also hybridize with I. usambarensis if it was not a distinct species from I. walleriana. However, there could be genetic factors responsible for the failure to hybridize. Seed was obtained from the I. usambarensis x I. gordonii cross, I-0201 x I-0301, but the germinated seedlings died at the cotyledon stage and further attempts at germination or hybridization failed. Therefore, it is possible that a hybrid may be obtained after more attempts.

Horticultural importance

Some of the species used in this study could have horticultural potential. Because I. walleriana is grown so extensively, new varieties are always welcome. The crispate margined petals of I. usambarensis could provide a refreshing change from the entire-margined petals of current I. walleriana cultivars. There should be no problem introducing I. usambarensis into existing cultivars because it forms fertile hybrids with I. walleriana.

Impatiens gordonii is not recommended as a cultivar because it is not a prolific flowerer. Our hybrids with I. walleriana were not very free-flowering either, but Arisumi (1980a) found that his were. Since some of the hybrids had very pretty flowers, especially the hybrid I-0301 x I-0111 (Figure 4.6), it may be worthwhile to backcross the hybrid to the I. walleriana parent. The colour of the I. gordonii x I. walleriana flowers tended to be closer to the blue part of the spectrum than the original I. walleriana parent, thus I. gordonii may be useful in developing a blue-flowered impatience cultivar.

Zinov'eva-Stahevitch (1981) lists I. flaccida as a conservatory ornamental but it may also have good potential as a bedding plant. It is very floriferous with pretty lilac or white flowers (Figure 4.4). Lilac flowers are not often seen in I. walleriana, so this could be a new colour. Both accessions were fairly resistant to spider-mites, but susceptible to aphids. It is also possible that I. flaccida var. alba was at one time under cultivation in 19th century England (Trimen and Hooker 1893).

Impatiens platypetala and I. platypetala var. aurantiaca have already been incorporated into the New Guinea Impatiens breeding program in the United States (Pasutti and Weigle 1980; Weigle and Pasutti 1979). The commercial cultivar 'Tangerine' is a cytotype of I. platypetala var. aurantiaca, but it is extremely susceptible to spider-mites, as are all strains of I. platypetala var. aurantiaca, and their offspring. I. platypetala may also have potential as a bedding plant.

Impatiens pseudoviola could be a good groundcover, because of its non-erect habit and pretty, delicate flowers. However, this species is also very susceptible to spider-mites.

Zinov'eva-Stahevitch (1981) and Grey-Wilson (1977) list I. sodenii as a conservatory ornamental. It is a gorgeous plant with attractive foliage and large flowers. The original two accessions of I. sodenii (I-0701 and I-0702) had fairly white flowers, but a cross between them produced a plant with lovely pale lilac flowers. Unfortunately, this species must be fairly large before it will flower, so it should be grown in a habitat such as a greenhouse or the southern United States where it would not suffer frost damage. Its potential as a houseplant is limited by its high humidity requirement. I have noticed that black spots on the underside of new leaves is a manifestation of spider-mite damage; even though the spots do not resemble spider-mite damage, and no mites seem to be present.

Impatiens harlandi has one of the prettiest flowers of all the accessions. It is a floriferous compact plant, but it has limited

horticultural potential because even in a greenhouse, it dies whenever conditions become less than ideal.

The Cucumber Mosaic Virus that infected some accessions and hybrids could have grave economic consequences. Most of the wild I. walleriana accessions were moderately to severely affected, and if the commercial cultivars are susceptible, the Impatience industry could be in a lot of trouble. Because there are only a few cultivars available, the commercial gene pool must be fairly limited, and Herold (1964) did isolate a Cucumber Mosaic Virus from I. sultani (=I. walleriana) plants growing in a garden in a housing estate in Venezuela. Cucumber Mosaic Virus also has a wide host range and is easily transmissible (Francki et al. 1979), so other plants growing in a garden could be at risk.

Chapter 8

CONCLUSION

From a total of 50 attempted crosses among nine species, hybrids were obtained between three species: *I. walleriana* x *I. gordonii* (and the reciprocal cross), and *I. walleriana* x *I. usambarensis* (and the reciprocal cross). However, it is considered that the ovule-culture technique of Arisumi (1980b) could be exploited to provide many more hybrids.

Both the *I. walleriana* x *I. gordonii* and the *I. walleriana* x *I. usambarensis* hybrids had regular meiosis in the vast majority of cells, but meiosis in plants of the former cross was more irregular than in those of the latter cross.

Because the meiotic irregularities observed in the two types of hybrids were very similar to those observed in the *I. flaccida* var. *alba* accession, the status of *I. gordonii* and *I. usambarensis* as species was examined. *I. gordonii* was considered to be a distinct species from *I. walleriana* because of the clear-cut morphological differences between the two species, and the higher number of meiotic irregularities observed in the *I. walleriana* x *I. gordonii* hybrids.

On the other hand, *I. usambarensis* was considered to be an ecological species of *I. walleriana* because the *I. walleriana* x

I. usambarensis hybrids had fewer meiotic abnormalities than the I. walleriana x I. gordonii hybrids. It was also very difficult to distinguish I. walleriana and I. usambarensis on a purely morphological basis. Because the two taxa are usually found in different habitats, I concluded that they may be ecological species.

Since both I. gordonii and I. usambarensis hybridize easily with I. walleriana, their species integrity could be threatened by the introduction of I. walleriana to their range. This has already happened in the case of I. usambarensis (Grey-Wilson 1980a).

However, the facility with which I. gordonii and I. usambarensis hybridize with I. walleriana could be very useful in the development of new cultivars in an I. walleriana breeding program. The I. flaccida accessions may have good potential as bedding plants, and the I. pseudoviola accessions may have possibilities as a ground cover cultivar.

The Cucumber Mosaic Virus that attacked most of the I. walleriana accessions, and many of the hybrids, could have grave economic consequences for the Impatience industry because this virus has a wide host range and is easily transmissible. Further studies to investigate the potential severity of the problem should be undertaken.

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